

# REVIEW ON AGRICULTURE AND RURAL DEVELOPMENT

SCIENTIFIC JOURNAL OF THE UNIVERSITY OF SZEGED, FACULTY OF AGRICULTURE  
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## GENETIC PARAMETERS FOR FITNESS TRAITS OF THE HUNGARIAN FLECKVIEH

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### ABSTRACT

Genetic parameters were calculated and breeding value evaluation was introduced for heifer and cow fertility, calving ease and stillbirth. Regarding fertility traits, the number of inseminations to conception (NINS) and non-return rate until 56 days (NR56) of heifers, NINS, NR56 and days open of cows were evaluated by REML procedure. The  $h^2$  of the heifers' two traits were 0.006 with a very strong genetic correlation (-0.95) between them. The  $h^2$  for cow fertility traits varied between 0.018 and 0.041. Due to the low correlation between heifer and cow fertility ( $r=0.14$ ), it is recommend that selection be based on both age groups. For calving ease and stillbirth variance components estimation and breeding value prediction threshold model. The heritability of heifer direct calving ease was 0.070, the maternal heritability was 0.226, the cow direct and maternal heritability was 0.034 and 0.135 respectively. An antagonism were observed between direct and maternal calving ease both for heifers (-0.635) and cows (-0.453). The correlation between heifer and cow calving ease was 0.2, which necessitates a separate breeding value evaluation, and different use of bulls for heifers and cows. For stillbirth, direct  $h^2$  was 0.047, the maternal  $h^2$  was 0.053 for heifers' stillbirth rate. The same parameters were 0.019 and 0.029 for cows. The genetic correlation between calving ease and stillbirth was 0.71 for heifers, and 0.43 for cows. Despite the decreasing tendency in stillbirth breeding value over the years, the inclusion of the trait in selection index is advisable for economic and animal welfare consideration.

**Keywords:** Hungarian Fleckvieh, fertility, calving ease, stillbirth

### INTRODUCTION

Fertility is one of the most important economic traits independent of the type. In dairy herds the economic value follows milk yield and the tendency for mastitis (SUN et al., 2009). The selection index for the Austrian Fleckvieh includes fitness traits with 46% weight alongside the 38% and 16% weights for milk yield and beef yield, respectively (FUERST and GREDLER, 2009). Selection for high milk yield has resulted in the deterioration of fertility (STEFLE et al., 1995, BANOS et al., 2004), which is about 3% by generation – a tendency that cannot be maintained (THALER, 1998). VAN RADEN et al. (2004) showed a 0.35 negative genetic correlation between fertility and milk yield. It is impossible to maintain the same level of fertility, or improve it without selection.

Dystocia causes economic loss primarily during the first calving as a result of stillbirth, veterinary costs after calving, prolonged ovulation, or other health reasons. MATURANA et al. (2007) calculated a 10% rise in replacement costs as compared to easy calving, which appears in shorter functional life span. The majority of veterinary costs is related to dystocia, which MCGUIRKE et al. (2007) calculated as £110 for calving assistance, and £350-400 for operations.

Stillbirth is defined as either a calf born dead or calf death within 48 hours after calving (PHILLIPSSON et al., 1979). Half of the cases derive from dystocia, one third from growth disorders, and other yet unknown reasons (BERGLUND et al., 2003). Not only does stillbirth



mean the loss of calves but the involution of cows is disorderly after stillbirth (STEVENSON and CALL, 1988), they ovulate and conceive later, have a higher risk for culling (+41%) or death (BICALHO et al., 2007). The milk yield of such cows is less during the first 60 days of lactation (BERRY et al., 2007a). In the USA, the occurrences of stillbirth increased from a 9.5% ratio in 1985 to 13.2% by 1996, which causes 125 million dollars loss in the dairy sector, therefore the replacement costs increased by 76 million dollars during the period mentioned (MEYER et al, 2001a).

## MATERIAL AND METHOD

Reproduction data collected by the Hungarian Fleckvieh Association between 2000 and 2009 were evaluated. Several aspects were observed for the validity and model harmonization of the data. Fertility measures analysed for heifers were NR56 and NINS, for cows were NR56, NINS and days open. If the female is recorded as reinseminated within 56 d of its first recorded insemination, then it is recorded as a failure (coded as 0). If the female does not have a second recorded insemination or its second insemination is greater than 56 d from its first recorded insemination, then it is recorded as a success (i.e., the female did not return to service, coded as 1). The genetic parameters for the fertility traits were estimated by the VCE6 software by REML procedure (GROENEVELD et al., 2008).

The breeder renders a score from 1 to 5 for the calving ease. Table 13 shows its definition and frequency. Calvings with codes 1 and 2 make up 85.56% and 93.3%, for heifers and cows respectively. In the course of our calculations codes 4 and 5 were combined due to their low occurrence. Data up to the 7<sup>th</sup> calving were considered, which meant 97.7% of the data. The average number of calvings was 2.73. We considered calvings in which the gestation length was at least 260 days, but no more than 300 days. Cases of abortion were excluded. The calving ease and stillbirth traits were evaluated by threshold model using the TM software (LEGARRA et al, 2008).

## RESULTS

The genetic parameters of fertility measures are shown in Table 10. The estimated  $h^2$  of two heifers' reproductive measures was 0.006, the genetic correlation between them is very close to 1 (-0.96), which means the two traits describe identical phenomena and are exchangeable. The  $h^2$  of fertility measures for Hungarian Fleckvieh heifers is higher than that of the Irish Holstein Friesian (0.000), and lower (0.014) for the Austrian Fleckvieh (GREDLER, 2008). Such low heifer heritability questions the inclusion of the fertility data for this age group into breeding value estimation and selection. All the  $h^2$  measures for cows (0.018-0.041) are consistent with those of the Austrian Fleckvieh (GREDLER, 2008). Due to the very high correlation between the number of inseminations and non-return rate (0.94), it is sufficient to include one of the two measures. Since the non-return state is more easily available, on day 56 after the first insemination, it allows earlier selection than the total number of inseminations. The  $h^2$  of days open is almost four times higher than the values calculated for the other two measures, therefore to apply it into selection is justified as it enhances reliability, and correlates with the other two measures on medium level.

KOMLÓSI and HÚTH (2010) evaluated the breed's genetic parameters of calving ease with a linear model in an earlier study. The direct  $h^2$  value of calving ease was found 0.048, the maternal  $h^2$  was 0.048. In the present study, the heritabilities for heifer calvings by



threshold model were 0.07 and 0.23 (Table 1). The higher values estimated by the threshold model are also supported by WELLER et al. (1988) and STEINBOCK et al. (2003). We calculated higher  $h^2$  values for cows' calving ease with the threshold model (0.03 and 0.13) as well, which was lower than in the case of heifer calving in a way similar to the linear model (Table 2). The correlation is negative between the direct and maternal calving ease; it is 5% for heifers and cows alike, and differs from zero on the probability level of 0.05. The direct-maternal correlation is negative in the majority of cases reviewed, which indicates antagonism. MEIJERING (1984) claims that calves born with ease have smaller weight, more favorable frame morphology, in cow age, however, they calve with difficulty. The repeatability value of the calving ease is 0.22.

**Table 1. Genetic parameters of calving ease and stillbirth calculated from heifers' calvings**

Trait	Parameter	Posterior mean	95% confidence interval of estimate
calving ease	direct $h^2$	0.070	0.068- 0.071
	maternal $h^2$	0.226	0.224-0.229
	$r_{\text{gdirect-maternal}}$	-0.635	-0,649 - -0,621
stillbirth	direct $h^2$	0.047	0.045- 0.049
	maternal $h^2$	0.053	0.051-0.561
	$r_{\text{gdirect-maternal}}$	-0.363	-0.400 - -0.352
calving ease-stillbirth	$r_{\text{gdirect-direct}}$	0.711	0.654-0.783

**Table 2. Genetic parameters of calving ease and stillbirth calculated from cows' calvings**

Trait	Parameter	Posterior mean	95% confidence interval of estimate
calving ease	direct $h^2$	0.034	0.031- 0.038
	maternal $h^2$	0.135	0.106 -0.167
	$r_{\text{gdirect-maternal}}$	-0.453	-0.549 - -0.357
	repeatability	0.220	0.218-0.222
stillbirth	direct $h^2$	0.019	0.017- 0.021
	maternal $h^2$	0.029	0.028-0.030
	$r_{\text{gdirect-maternal}}$	-0.451	-0.483 - -0.419
	repeatability	0.024	0.023-0.025
calving ease-stillbirth	$r_{\text{gdirect-direct}}$	0.431	0.415 – 0.447

## CONCLUSIONS

Low heifer heritability questions the inclusion of the fertility data for this age group into breeding value estimation and selection. All the  $h^2$  measures for cows (0.018-0.041) are consistent with those of the Austrian Fleckvieh. Due to the very high correlation between the number of inseminations and non-return rate (0.94), it is sufficient to include one of the

two measures. Since the non-return state is more easily available, on day 56 after the first insemination, it allows earlier selection than the total number of inseminations. The  $h^2$  of days open is almost four times higher than the values calculated for the other two measures, therefore to apply it into selection is justified as it enhances reliability, and correlates with the other two measures on medium level.

Calving ease is in 3-7% of direct, in 13-23% of maternal genetic origin. The maternal effect on the trait is more considerable than that of the direct effect. Antagonism between the direct and maternal gene groups is of medium level. If we explain the negative correlation with birth-mature weight, and birth-mature frame morphology, then this phenomenon necessitates data collection and evaluation of birth and mature weight as well as shoulder and pelvic width. In the Australian beef breeding practice, selection for calving ease resulted in slim calves whose mature pelvic bone is narrow. It can be recommended for terminal breeds only. In the Hungarian Fleckvieh a compromise needs to be established (medium calf weight, medium pelvic size, medium cow weight), which requires data collection. In the absence of data, it is possible to observe the combined breeding values of direct and maternal calving ease.

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## ANIMALS ARE FED, AND WE PEOPLE, ARE EATING

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One of the most essential conditions of the human and animal life, besides breathing, is nourishment (including water!), that has to be intake every day. How simple and clear! As someone would imagine, who never had (any) animals yet, who just goes into the store and buys the "daily".

This presentation is a brief description, and guaranteed not complete, attempts to show the characteristics of feeding farm animals (in particular those identified as omnivorous pigs) and of the (also omnivore) human diet. The basic idea of the compilation is to review some important nutritional characteristics of healthy humans and animals – always compared simultaneously –, and to explain it with some extras.

The first starting point of the presentation is the 2008. XLVIth Act about the food chain, and its official control, and its 7th §: "...feeds cannot adversely affect neither the productivity of the animals nor endanger or harm the animal, directly or indirectly the human health"

Another starting point is that

- we are not eating because we would like to cover our nutrient requirements, but for the pleasure of the white table!
- our eating habits are part of our national culture!
- but it should be kept in mind that eating is a basic biological need!

**A small glossary:** 'fattening' animals means that they are raised for meat production / rearing. "Gain" in practice means all the weight (mass) gain while in the research there is the protein and/or fat gain as well. The "fatty" animal is "cutting-ripe" with the desired body composition. In the human practice "weight gain" and "obesity" always mean "fat gain", and the weight gain is by analogy to the "weight" change.

**Why do we feed, and why do we eat?** Basically, to increase the production of animals (except for pets), including covering their nutrient requirements, according to the well-being (animal welfare) and the environmental protection requirements. The aim of human diet is basically enjoyment (deliciousness) and covering the nutrient requirements is only a secondary target, but health cannot be ignored. Exceptions are mothers and young children, and some special situations (e.g. sports, military).

This simple activity (i.e. feeding and nutrition), starts highly complex processes in the organs that are tested and dealt with multidisciplinary.

The animal and plant **genetics** are production-oriented and continuous (such as plant and livestock breeding, crosses/hybrids, GMOs, performance tests, offspring control, etc.), while human genetics deal particularly with disease forecast, genetics, and they face a tremendous growth with the introduction of "gen surgery".



**The feeding/nutrition science** deals with the refinement of the animal nutrient requirements, the feed processing and feeding technologies. The human nutrition and the food science works out special/diet plans, specifying the nutrient supply, developing recommendations for the meals of the different age groups.

The **anatomy** of the gastrointestinal tract in pigs and humans is very similar. There is a "monogastric" simple stomach, the size and function of various intestinal sections are almost identical (unlike the ruminant gastrointestinal tract, where the stomach is complex and has four compartments).

**System of feeding and nutrition:** feeding animals is on strictly cost/benefit basis (approximately since 1970 it is computerized – using a linear optimization, with biphasic simplex algorithm), according to age and utilization of animals, with minimizing the price of feed or the cost of feed in respect to the food safety and animal welfare aspects. The nourishment of people is mostly at "luxury" level (exceptions are: the "institutional" (public) food, for example military, schools, etc, and the hunger zones), taking food safety into account. The calculation is usually done by age (certainly not in the household, but in the institutional meals (at least in theory)).

**Compilation of the feed/food recipes:** The composition of feed is rational, on price or cost minimum, basically on age or utilization and quality needs according to nutrient requirements. Our recipes are "irrationally" put together, according to the hedonic value, habits, fashions and trends, and many times on misconceptions and/or ignorance, more recently, on the "conscious" (?) based diet! Part of this "irrationality" is the inadequate knowledge of nutrition – which is not new at all.

**The nutritional value when calculating the nutrient content of** in case of animals is almost exclusively based on *in vivo* or *in vitro* specific digest ratio, which does not mean the actual digestibility, but is closer to it than the chemical analysis of several foods, on which our foods are still reported. This is especially significant, among others because of the different effects of heat treatments on the (protein) digestibility (e.g. gently drying the corn, and frying the meat!). The pig's **energy** requirement, and accordingly the energy content of feed are calculated as DE (digestible energy) or ME (metabolizable / transformable energy) (possibly NE (net) energy). Food energy content and the recommended energy intake are always calculated with the GE (i.e. gross energy "heat of combustion") value. Another significant difference is the evaluation of **proteins**. In case of feeds the crude protein ( $N \times 6.25$  i.e. because it is the easiest and cheapest to determine), or the digestible protein (and sometimes a true protein) data and the crude protein together with fecal or ileal (*in vivo*) digestible and/or the available (*in vitro* "digestibility") amino acids are given. In contrast with that, the protein content of the foods is usually only given as total protein (i.e.  $N \times 6.25$ ).

The basis for the calculation of the feed composition is the DE/amino acid (lysine) ratio, while in the food, the energy and protein content are calculated separately. It is well known that the unwanted fatty gain of pigs can only be avoided if the intake of energy and lysine (and other amino acids expressed as percentage of lysine) is in certain proportions.

**The carbohydrates** in the feed are recorded as the so-called (digestible) N-free extracts  
[calculated as: organic matter – (crude protein + crude fat + crude fiber)]



only rarely declaring the starch content separately. In the food usually only the total carbohydrate content counts, and recently the concept of resistant starch (which is a non-digestible carbohydrate that functions as a fiber) has appeared.

Although fibers are counted separately, they are also carbohydrates. In case of feed these are crude fibers and/or digestible fibers (cellulose, hemicellulose, and lignin = structural fiber), in food dietary fibers (partially or completely resistant to enzymes). Despite the apparent similarities, the concept behind them is different and their chemical analysis is different too.

The **ash** is a residue if the feed or food material is burned, which means the content of the inorganic material. In feeds crude ash is counted, where the macro- and microelements (i.e. minerals) are included. In response to requirements for these (e.g. Ca, P, microelements), regular and complete supplementation are in the feed for all animal species, based on age or utilization of them. In case of food we are talking only about "minerals", and supplements are mainly up to individual demands. Both supply and environmental reasons are considered when the efficiency of utilization of minerals (such as available or digestible phosphorus) in feeding is calculated.

Feeds have also a regular and complete **vitamin** supplement, with the same system as the minerals and the human diets will only be supplemented on individual needs too.

There is a lot of misinformation in the public opinion about the drug content of the feed, in turn in raising and farming of livestock, **using preventive drugs is strictly prohibited!**

Many people do not realize that the potential range of feed and food are almost the same (practically interchangeable!), and this is regulated by the law cited above. (An example: as feed wheat is used as coarse meal or bran, as food it is used as miscellaneous quality ground into flour or coarse meal, and the bran is also eaten as well).

Either feed or food/meal is in some way, to a greater or lesser extent, it has to **be prepared**. It can be done in a feed mill or on the farm (feed-kitchen), in the food industry or in an amateur (household) or a professional kitchen.

**Feeds are basically used in their original form**, but in many cases prepared with some sort of technology, **foods are basically processed** but sometimes used raw (original form). The reason for feed processing is achieving higher transformation efficiency, for food processing, it is increasing enjoyment for example by heat treatment. Cooking/baking our foods, as excavations show, have been used for several thousands of years, and are not an invention of modern times!

Although **the processing technology is nearly the same, but the utensils used differ significantly**. In the feed processing there are different possibilities e.g. reduction of feed particle size or heat treatment (e.g. extruding, expanding, flaking, micronizing), pelleting (in this case grain size is increased) or moistening, etc. The technology of food preparation is the classical cooking, steaming, baking in oven, roasting in grill, etc.. The tools used are: the classical "stove", microwave "oven", the steamers, the hot air "ovens", and there are classical and "new" (?) methods (e.g. cooking in vacuum/sous vide, baking at low temperatures, etc.) as well.

To sum up the above introduced facts: feeding is always based on the latest scientific results, on strict cost/benefit basis, paying maximal attention to animal protection, animal welfare, food safety and environmental aspects. The human nourishment and eating (taking food safety into account) is realized basically on the hedonic value, but it is a typical and



important part of national culture. Therefore, it should never be forgotten that human nourishment is different and more:

„...believe me, a meal prepared without heart, mood and loving care is like the person who made it. I would like to hand over this love, this mood and this heart to everybody, who prepares a dish by cooking, baking, roasting or frying for his/her family, guests or customers; no matter what the circumstances are: either in a small or large kitchen, like a poor or a rich man, in a modest pot or in a shiny kettle, on an old or on a modern stove, from the cheapest or from most expensive material in order to give culinary delights...

And all this is to make...

..... with eyes open, paying attention and with strong nerves, always keeping one eye on mischievous sprites not to let them spoil the food prepared with much efforts, that we can give to our guests with a good appetite that pure pleasure of life which can only be expected at the table.” (KÁROLY GUNDEL, 1941)

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## GOLD-RESERVES OF THE HUNGARIAN AGRICULTURE

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### ABSTRACT

The performance of Hungarian agriculture got into a depressive condition regarding its potential opportunities. Despite the excellent natural conditions the significance of crop production and animal breeding declines, in certain branches it has reached critical levels. During the past years sugar beet yield was only 10% of the potential. Potato, vegetable, fruit and grape productions produced 24%, 51%, 19% and 28% of the potential yields, respectively. Hog production realized 38%, milk production gave 59%, while egg production provided 58% of the potential production levels. This agricultural depression has multiple reasons. The author does not deal with revealing the reasons of production decrease. He gives recommendations for getting out of the depression, which he calls as the gold-reserves of the Hungarian agriculture.

**Key words:** gold crown, land tax, land appraising system, crop production, animal breeding

### THE AGRICULTURE IS HUNGARY'S GOLD RESOURCE!

There is an old phase: „The one who has gold has always money.” (FOUR GATES)



Figure 1. The Change of the Price of Gold and the Value of Money  
(FOUR GATES, 2010)



## THE PERFORMANCE OF HUNGARIAN AGRICULTURE

The performance of Hungarian agriculture is based on its excellent natural conditions. Primarily land quality and climatic conditions determine its production. Besides the excellent natural values economic conditions have an influence on the potential of agriculture. In order to reach the upper limit of potential opportunities fortunate coincidence of ecological and economic possibilities and their utilization are necessary.

The upper limit of the performance of agriculture may be well quantified. The long-term time-series database of Central Statistical Office reflects the maximum and minimum of performance of certain branches. This database encompasses five decades at present, such as the period between 1960 and 2010. During this period natural conditions may be considered as unchanged; however, the economic conditions may be classified into four well-separated periods. The period between 1960 and 1967 is characterized by the formation of large agricultural concerns and central state control of farms. The period between 1968 and 1989 was suitable for the independent possibilities of large-scale farming. The period between 1990 and 2003 lasted from the change of regime till the EU accession. The period of EU regulation has started since 2004.

Let us review the upper limit and bottom of production in major agricultural branches. Potential (upper limit) is the production volume of years, which has been reached once since 1960. Bottom of production are the lowest elements in the time series. *Table 1* indicates the upper limit of potential and bottom of production in crop production.

**Table 1. Performance of Crop Production**

Denomination	Performance of crop production	
	Upper limit	Bottom of production
Cereals	16,841 million tons (2008)	6,210 million tons (1961)
Wheat	7,392 million tons (1984)	1,624 million tons (1963)
Corn	9,050 million tons (2005)	2,737 million tons (1961)
Sugar beet	5,867 million tons (1991)	0,573 million tons (2008)
Oil seeds	2,216 million tons (2008)	0,100 million tons (1960)
Potato	2,013 million tons (1969)	0,488 million tons (2010)
Vegetable	2,248 million tons (1988)	1,144 million tons (2010)
Fruit	1,935 million tons (1982)	0,360 million tons (2007)
Grape	1,047 million tons (1982)	0,295 million tons (2010)

The upper limit of potential performance as well as bottom values may be characterized by the number of animals and the quantity of animal products (*Table 2*).

**Table 2. Number of Animals and Performance of Animal Breeding**

Denomination	Performance of Animal Breeding			
	Upper limit		Bottom values	
Cattle	2,017	million cattle (1974)	0,682	million cattle (2010)
Pig	9,844	million pigs (1983)	3,169	million pigs (2010)
Sheep	3,180	million sheep (1982)	0,858	million sheep (1997)
Poultry	72,049	million poultry (1970)	31,244	million poultry (1999)
Slaughtered animals	2,418	million tons (1984)	1,070	million tons (1960)
Hog	1,460	million tons (1984)	0,553	million tons (2010)
Cow's milk	2,787	billion liters (1988)	1,641	billion liters (2010)
Hen egg	4,748	billion eggs (1978)	1,835	billion eggs (1962)
Wool	12,762	thousand tons (1982)	2,959	thousand tons (1997)



Regarding the recent results of crop production (relating to 2010) the products of intensive cultures are critical. **During the past years for example sugar beet production was only 10% of the potential. Potato, vegetable, fruit and grape productions produced 24%, 51%, 19% and 28% of the potential yields, respectively (Table 3).**

**Table 3. Recent Performances of Crop Production**

Denomination	Performance of crop production	
	2010	Bottom of production
Cereals	12,262 million tons	6,210 million tons (1961)
Wheat	3,745 million tons	1,624 million tons (1963)
Corn	6,985 million tons	2,737 million tons (1961)
<b>Sugar beet</b>	0,819 million tons	0,573 million tons (2008)
Oil seeds	1,604 million tons	0,100 million tons (1960)
<b>Potato</b>	0,488 million tons	0,488 million tons (2010)
<b>Vegetable</b>	1,144 million tons	1,144 million tons (2010)
<b>Fruit</b>	0,766 million tons	0,360 million tons (2007)
<b>Grape</b>	0,295 million tons	0,295 million tons (2010)

The main problem in animal breeding is caused by the decrease in animal number, thus **hog production realized 38%, milk production gave 59%, while egg production provided 58% of the potential production levels (Table 4).**

**Table 4. Recent Performances of Animal Breeding**

Denomination	Performance of Animal Breeding			
	2010		Bottom values	
Cattle	0,682	million cattle	0,682	million cattle (2010)
Pig	3,169	million pigs	3,169	million pigs (2010)
Sheep	1,181	million sheep	0,858	million sheep (1997)
Poultry	42,213	million poultry	31,244	million poultry (1999)
Slaughtered animals	1,329	million tons	1,070	million tons (1960)
Hog	0,553	million tons	0,553	million tons (2010)
Cow's milk	1,641	billion liters	1,641	billion liters (2010)
Hen egg	2,732	billion eggs	1,835	billion eggs (1962)
Wool	4,070	thousand tons	2,959	thousand tons (1997)

**This agricultural depression has multiple reasons. Revealing these reasons of production decrease is not a part of the study.** Data indicate that upper limit of the production of crop production branches share among the period of large-scale farming and periods after the change of regime and EU accession. The lower level of production and the low numbers concentrate on the period of state controlled farms as well as period after joining the EU. The animal number and the upper limit of production were realized in the period of independent large-scale farming, while bottom of production evolved after the change of regime and EU accession. During these periods the control of agriculture, and the regulation of production happened by altering tools. Prices, subsidies, credits, taxation approached to agriculture in different ways. Their correlations may be detected by results. **Within the presentation I suggest recommendations, mainly for changing the system of taxation, for getting out of the depression, which I call as the gold-reserves of the Hungarian agriculture.**



## **CHANGING THE TAXATION SYSTEM OF AGRICULTURAL PRODUCTION**

The taxation system of agriculture is attacked by many people in different ways. Quotes are highlighted from two media presses as examples.

„Today the black turn-over of food industry is estimated 1500 billion HUF. Its lost VAT-content is about 400 billion HUF. We live in a crisis; its moment would be a possibility for carrying out measures for the sake of the commonweal to aim at reaching order and approaching more moral version of profit and burden shares. Even saint cows may be considered as well, such as small and family farmers who do not want to confess their production, millers holding to ransom, or foxy bakers. Furthermore, even 600-700 thousand land-owners, who have nothing to do with farming but collect annually untaxed land rent fees of 120 to 150 billion HUF legally at present” (TAMÁS, 2011).

„One of the biggest problems of agriculture is the black market sharing 20 to 40% for years. This makes the situation of larger firms and honest small-scale producers more difficult, who are not capable of selling their goods without giving bills. Black goods are cheaper by 25% than legal products. It is impossible to catch up this price difference by improving the efficiency. It means that who works honestly, has to cope with such a disadvantage. Many gave up, every second sty has become empty for today.

Moreover, crop production even shows these signs: 30 to 40% of cereal trade belongs to the black market, by this taxes of 20 to 30 billion HUF in every year do not get into the state budget, strengthening the mafia specialized for VAT-fraud. Similar numbers are present in meat industry, poultry production and vegetable and fruit sector. Annually approximately 100 billion HUF is estimated staying away from the state budget.

It is a step for the change of fortune. Reverse VAT paying will be introduced in agriculture in the future. The black market may be reduced; tax revenue may be increased by 100 billion HUF. Now it is not worth cheating because there is no tool to do so. It is imaginable that fragmented production may be harmonized again, price advantages may be reached by common selling and purchasing, which means that the whole agriculture may be based on plans in a better way” (O. HORVÁTH, 2011).

Taxation of agricultural production is extremely difficult reflecting a bureaucratic way. Tax returns of producers are complicated; it is almost an insoluble task from small-scale farmers to large farms without tax advisors. The majority of farmers look for the possibilities to circumvent tax regulations or try to evade the tax, one hand because of Hungarian mentality, on the other hand for reducing costs. The profitability in majority of branches cannot cope with further increase of costs.

230 year before, our emperor, Joseph II had a land-register worked out, a taxation system relating to the increment of territories, which though he withdrew before his death further developed. Land tax, the taxation system based on the net income of each parcel of land registered in the land cadastre (later converted to gold crowns) was developed by 1875 (CompLex, 2012).

### **„Act VII of 1875 on Land Tax Regulation**

#### ***Chapter I. General Decisions***

1. § Net income of every land parcel in the territory of the Hungarian state should be investigated again and a general land tax register is made for the sake of proportional taxation of land.

## **Chapter II. The Calculation of Taxable Net Income of Land Parcel**

8. § The net income of a land parcel equals with the difference between the value of permanent middle yields and real costs regarding common farming.

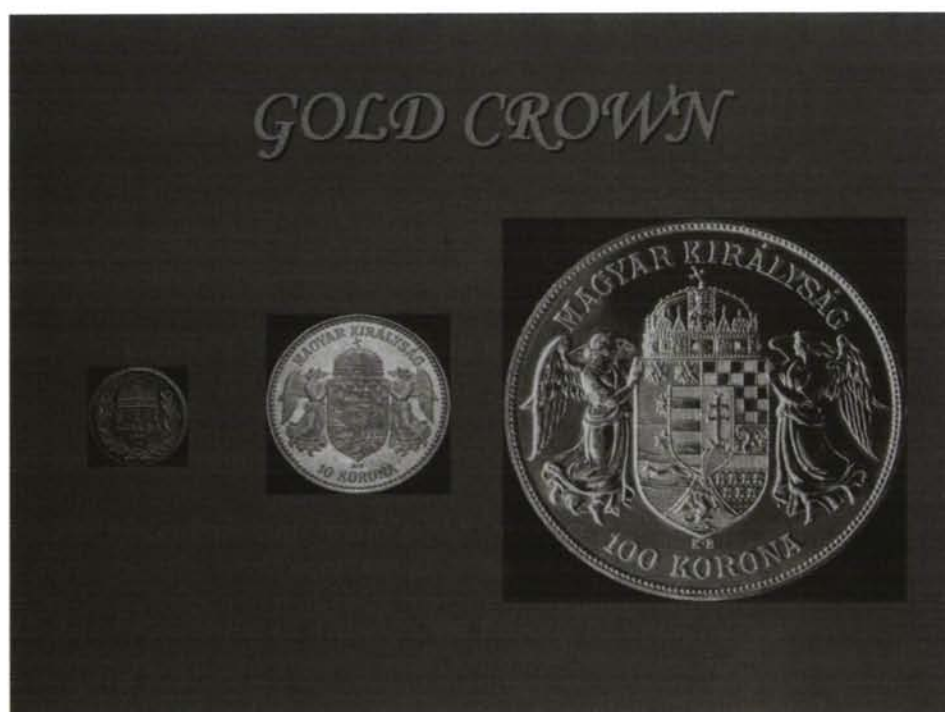
### **The Ratio of Land Tax**

2. § The land tax is imposed in same ratio after net income of every land parcel determined by a separate tax law.

7. § The land tax is determined for the parcel of land and is paid by the actual owner.”

**The ratio of land tax is a percentage determined in a separate tax law of yearly net income** (registered net income = production value – production cost) **of a parcel of land** (cadastral acre = 5755 m<sup>2</sup>). (Its ratio is 29.77% in Hungary, while in Transylvania it is 22%.)

The **gold crown-value** as the synonym of the registered net income evolved due to the conversion of the monetary system of Austro-Hungarian Monarchy after introducing the gold crown (*Figure 2*). Its name connects to coinage of gold money and to different denominations of gold crown coins (1892-1918).



**Figure 2. 10 and 100 Gold Crowns** (WIKIPÉDIA, 2012)

**Introduction of land tax again on the basis of gold crown-value is one of the gold-reserves of the Hungarian agriculture!**

I would mention in advance that I would not like to make the situations of land owners and farmers harder by recommending the introduction of another tax, but exchange all of the others by only one tax. This may decrease bureaucracy, help selling agricultural goods, enhance rural employment and improve the profit of branches.

Those paying land taxes might enjoy different privilege during selling their products. The obligation to give bills might be ceased, farmers should not pay for places in markets, might sell their goods freely in public places being not prohibited, might get selling



possibilities in supermarkets etc. They might hire seasonal workers without any limitations. In case of controllers, it would be enough to show a card justifying the fact that the tax was paid. The number of privileges could be enhanced in several ways.

The introduction of land tax would not be popular first. It would not be easy to accept the profit-based taxation based on land quality and location again. One of the first steps is to modernize the gold crown-value or to complete the already started 100-point-land appraising system. Then thorough, sound, well-considered and harmonized series of measures should have to be taken for the sake of success.

The „Hatted King” tried but failed. But He also knew the rational solution: „...the big plain is not for dancing mirages being bored but for pouring cereals. And no water fowls should prevail on the Danube but well loaded ships should be in a hurry between East and West.”

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## THE AGRICULTURE OF AN ULTRAPERIPHERAL REGION OF EUROPEAN UNION: THE AZORES ARCHIPELAGO

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### ABSTRACT

The Azores archipelago is considered to be an ultraperipheral region of European Union, because some of the characteristics of their development, and some specific politics were applied in these regions. A specific European development program, Azores Rural Development Plan – PRORURAL – for 2007 to 2013, is supported by the European Agricultural Fund for Rural Development and is ongoing at this moment in Azores Archipelago.

The objective of this paper is to characterize the type of agriculture carried out in the Azores Region. It starts by a small introduction of the Azores according to the Statistical National Institute (INE) and its brunch SREA – Statistics of Azorean Region - and finally by the Farm Accountancy Data Network (FADN) Then it characterizes the agricultural farms and finally, it shows some relevant researches about Azorean farms, namely; (1) a classification of the Azorean farms and the type of objectives that can influence the decision-making of the Azoreans' farmers (2) the efficiency of dairy farms and (3) the analysis of the impact in the incomes' farmers.

In the Azores, agriculture is based on dairy cattle productions and the remainder agricultural productions are residual. The Azores' islands produce mainly cow's milk (in 2010 it represented 30% of Portuguese milk production), but also beef. And there products comes from quite different production systems ranging from extensive to intensive grazing systems.

The average agricultural area per farm is small (8.5 hectares) and the efficiency of the dairy farms is relatively low due in part to equipment amortization and is highly dependent of subsidies and - farms will not be economically sustainable if the subsidies were eliminated.

**Key-words:** Azores, Dairy milk, Decision, Efficiency, Grazing system.

### INTRODUCTION

The objective of this paper is to present the Azorean agriculture and some researches applied in this region, namely in what concern to the importance of subsidies in Azorean agriculture.

The Azores archipelago is a part of Portugal and is composed of nine islands distributed by three groups; the occidental group with the Corvo and Flores Island; the central groups with S. Jorge, Graciosa, Pico, Fail, Terceira islands, and the oriental group with S. Miguel and Santa Maria islands. The Azores archipelago is in located in the middle of Atlantic Ocean between the North American and Europe, being the Flores Island the most occidental frontier of Europe, where the Europe Continent starts. The geographical coordinates of the Azores are; latitude (extreme points) 39° 43'23''N and 36° 55'43"N and longitude (extreme points) east 24° 46'15" WG and west 31° 16'24" (SREA, 2010).

The total area of Azores is 2322 Km<sup>2</sup> (SREA, 2010). The biggest island is S. Miguel (32% of total area) and the smallest is Corvo (1% of total area). Pico and Terceira islands represent about 19% and 17% of Azorean total area. The Pico Mountain is the highest point of Portugal with 2351m high.



The resident population in the Azores in 2009 was 245435 inhabitants, but the most part (77.49%) live in S. Miguel (54.71% of the total inhabitants) and Terceira (22.78% of the total inhabitants). Corvo Island only has 500 inhabitants (0.20% of total). The population density, according (SREA, 2010), was in the 2009 about 105.7 inhabitant/km<sup>2</sup>, and ranges from 180.4 inhabitant/km<sup>2</sup> in S. Miguel Island to 29.2 inhabitant/km<sup>2</sup> in Corvo Island (similar to Flores Island – 29.4 inhabitant/km<sup>2</sup>). Terceira has the second highest population density - 139.7 inhabitant/km<sup>2</sup>. The unemployment rate, according to the same source, was in 2009, about 6.7%.

The gross domestic product of Azores, in the 2008, represented about 2.03% of Portuguese product and the annual average rate in the consumer prices was 0.8% in 2009 (SREA, 2010).

In 2009 the utilized agricultural area (UAA) of Azores, was 112 054 hectares (3.23% of total of Portugal) and comprised 13149 farms (4.8% of Portugal farms). The arable area is about 8.4% of the area and about 52.9% of the farms. The vegetable farms represents about 0.42 of total area and 54.4% of the total farms. The permanent crops represent about 1.9% of the total area and 47.3% of total farms. The meadows and permanent grassland is about 89% of the area and 65.5% of the holdings. This means that the most important culture in the Azores archipelago is the meadows and permanent grassland and the permanent crop and vegetable farms are marginal. The average agricultural area per hectare in Azores, in 2009, was 8.52 hectares per holding (SREA, 2011). In 2007, the average agricultural area per hectare in Portugal was 13 hectares and in Hungary 7 hectares ((Martins and Tosstorf, 2011)

The UAA per holding is 8.9 hectares, but they have some dispersion. The most representative (34% of total UUA) class size area is from 20 to 50 hectares and higher than 50 hectares (31.8% of total UUA). The holdings with 5 to 20 hectares represent 25.6% of total area. About 8% of holdings have less than 5 hectares (SREA, 2011).

In 2009 there was about 13 541 sole holders and 133 companies. The most part of farmers (84%) is on their own and a small part in a leasing regime or both (SREA, 2011). The annual working unit is 1.3, and the labor force is mostly familiar work (holder, spouse and other family members). There are 11 532 people working on agriculture and of these, 74.1% are men (SREA, 2011).

The main temporary crops production in Azores is cereals (maize-grain, maize-forage and potato). The main permanent crops are citrus fruits (orange and tangerine); fresh fruits (apple); subtropical fruits (pineapple and banana); nut fruits (chestnut) and vine for wine. There are also, others cultures, namely tobacco, sugar beet and tea (the only place in Europe with a relevant production).

The main agricultural production in the Azores islands is animal production, especially cow's milk production (540 199 225 liters per year). In 2010, it represented about 30% of Portuguese cow's milk production. But beef is the other important production in Azores. There were about 245 thousands heads of bovines in the Azores islands (SREA, 2011). The dairy cow represents 79.8% of the cows in the Azores. There are about 28.2 dairy cows per farm and 32 cattle per farm. The live stock unit per UAA in Azores was 1.71 in 2009. The cattle slaughtered in the Region, was mainly the cattle 50 385 heads accounting for about 11 565 tons. Pigs, sheep and goats and also aviculture are not relevant in the Azores archipelago.

### **Agricultural researches in Azores**

SILVA AND BERBEL (2006), in a previous research, defined types of Azorean farms from a panel data of 174 farms of The European database of Farm Accountancy Data Network of the Azores, Portugal. This study used cluster analysis, the Ward method. The results,



allowed the identification of three types of grazing systems of dairy farms as follows: 1) extensive grazing systems (less than 1.4 cows per hectare); 2) moderate intensive grazing system (1.4 to 2.4 cows per hectare); and 3) intensive grazing system (more than 2.4 cows per hectare).

SILVA *ET AL.* (2004) measured the Azores dairy farms technical efficiency by applying a non-parametric efficiency analysis to a panel data of 122 dairy farms from the Azores, Portugal for the year 1996. The analysis used DEA with constant and variable returns to scale models, with an input-oriented model approach. Two outputs (milk production and subsidies) and three inputs (agricultural area, number of dairy cows and variable and fixed cost) were considered relevant. The results suggested that the average technical efficiency is very low (66.4%) compared with published research data and only a few (7%) dairy farms were found to be efficient. The meaning of the average technical efficiency (0.664) is possible to produce the same amount of milk while saving approximately 33.6% of resources (or inputs).

One line of research in the Azores was the efficiency of farms. MAROTE AND SILVA (2002) had analyzed the importance of subsidies in the Azorean dairy farms efficiency from 1997 to 1999. The technical and economic variables of 82 dairy farms of the FADN (Farm Accountancy Data Network) were analyzed over the period of three years. The DEA (Data Envelopment Analysis) was the approach used to calculate the efficiency. The results shown that the subsidies were not so important in the dairy farms efficiency along the three years. The technical efficiency variable and constant returns do not present great differences between the model I (with subsidies as the output) and model II (without subsidies as the output). The number of efficient dairy farms was quite different and the decreasing subsidies seem to be compensated by the dairy production increase. In the Model I (two outputs and nine inputs) the average technical efficiency returns to scale (VRS) is high and very homogeneous (the standard deviation was less than 0.1). Its higher mean value was about 0.960 in 1999. The mean technical efficiency a VRS is higher than 0.950 in the three years, although it was possible that some farms increased its efficiency a VRS, as the minimum value confirms it (from 0.504 to 0.637). That means that these farms could produce the same using less inputs. The number of efficient farms goes from 62 % (51/82) to 71 % (58/82) and there were 34 farms that were simultaneously efficient in the three periods (they are about 41% (34/82) of dairy farms). In the model II (one output and nine inputs) the efficiency of the VRS was high along the period 1997 to 1999 and its value was about 0.943 and 0.953. Despite this, there were some farms that could improve their efficiency (the minimum value of efficiency ranged from 0.461 to 0.611). The number of efficient farms goes from 55 % (45/82) to 67 % (55/82). There are 28 farms which have always been efficient along the three years, but this value is superior in the model I.

SILVA *ET AL.* (2007) proposed to estimate the technical efficiency in agricultural grazing systems (dairy, beef and mixed) in Azores in the year 2002. This research used 184 agricultural farms of FADN- Farm Accountancy Data Network. DEA, a non-parametric methodology, was used to estimate efficiency, by means of DEAP software. The results have shown that the average technical efficiency in the dairy grazing system was 63.2% (CRS) and got higher (71.4%) in VRS and the scale efficiency was about 89.2%. In beef grazing system, the average technical efficiency (CRS) was 69.4% and VRS was higher (82.9%) and the scale efficiency was 84.2%. In the mixed grazing system, the average technical efficiency (CRS) was 89% and the VRS was higher (99.24%) and the scale efficiency was 89.8%. The mixed system is the "most efficient". In the dairy system grazing only 9.8% were efficient enterprise, nearly 11.1% were efficient farms in beef systems and about an half (46.7%) of the farms were efficient in mixed grazing systems.



The efficiency was generally higher in mixed systems when compared with the other two grazing systems (dairy and beef).

NONCHEVA *ET AL.* (2009) used the Canonical Correlation Analysis in variable selection to estimate the efficiency in Azores dairy farm. With this method, DEA models are used by PAR methodology to measure efficiency, making it possible to rank observations (Azorean farms) in terms of their dissimilarity to other observations in the data (other Azorean farms). This makes PAR appropriate to support public policies in agriculture sector in the Azores.

SILVA AND VENÂNCIO (2004) had used a stochastic frontier production (SFA) for three types of farms to estimate the efficiency and inefficiency of farms types. The Frontier Program allows the estimation of efficiency (model I) and inefficiency models (model II). The levels of efficiency are constant and similar along the time and their values are 82%, 93.2% and 85.1% respectively for the clusters A, B and C. The variables that cause inefficiency are subvention and equipment amortization. The variables more connected to efficient farms are lower rent fields, dimension and beef sales. The bigger farms seem more efficient, which was also observed by Hallam and Machado (1996) in the Portuguese farms from the north of Portugal.

In the Azores the subsidies are support by PRORURAL program approved by Commission Decision C (2007) 6162, on the 4th of December 2007. The PRORURAL has four center lines: 1) improving competitiveness of the agricultural and forestry sectors; 2) improvement of the environment and rural landscape; 3) quality of life in rural areas and diversification of economy; and 4) operation of LAGs, Acquisition of skills and entertainment in rural areas.

## **MATERIAL AND METHODS**

In this survey 30 farms from AAIT (Farmers Association of Terceira Island) for 2007 were analysed. This database takes a part of Farm Accountancy Database Network – FADN.

On average, the main characteristics, of the farms of this survey are: small dimension of farm is 18.4 hectares, they are familiar (one worker, the owner, working 1920 hours a year); with 42 animals per hectare (about 62.5% are dairy cows) and the intensification level is about 2.29 animals per hectare. Two farms have also fruit production (banana).

The variable costs per hectare are about 978.8€ and represent 64.1% of total cost. The fixed cost per hectare are 526.5€ and represents 34.5% of total cost. The remains, 1.5%, are cost taxes.

## **RESULTS**

The main costs of these farms are: concentrated feeding (38% of total cost); fertilizers (14.1% of total cost); equipment amortization (14% of total cost); land rent paid (7.8% of total cost); and fuel (7.3% of total cost). All other costs accounts for less than 5% of total.

In this research (2007) the subsidies contributed to the total farm production with a quarter – 25.3% of farmers' income. This value is quite similar to previous researches. It was showed that the importance of subsidies were 15.3% in the Azorean dairy farms of the total output (SILVA, 2001), MAROTE AND SILVA (2002) presents the amount of subsidies received from 1997 to 1999, and it was decreasing value (from 19,3% in 1997, to 15,9% in 1998, and to 11,8% in 1999). SILVA AND VENÂNCIO (2004) observed that the amount of subsidies in total product for Faial island farms were 16.3%, 27.5%, 22% and 20%



respectively to the years of 1996, 1997, 1998, 1999. That means for the amount of 10 000€ of farmers income, 2500€ were from subsidies, the same value presented for agricultural farms in 2004 (SILVA, 2004). The main important subsidies in Azorean agriculture were POSEIMA, ranging from 43.4 to 32.6% of total subsidies from 1998 to 2003, and compensated payments ranging from 24.6% to 22.8% from 1998 to 2003 (SILVA, 2004).

As can be seen in *Table 1*, the gross margin per hectare and year is about 17 208.4€, but if it was divided by twelve months, it would remain 1 434.0€, which means that it is an adequate income for the Portuguese reality. But if the gross margin without subsidies is considered, the value decreases, to 9 632.2€ per year and to 802.7€ per month. That means that if the subsidies are taken off then the income decreases 7 576.2€ per year and per 631€month.

The net margin per hectare was about 11 600.2€ per year and 966.7€ per month, and it is a satisfactory income in the Portuguese context. The net margin without subsidies decreased a lot – 4 024.0€ per year and 353.3€ per month, due not only to the amount of subsidies, but also to the increasing amortization cost. The net margin per month was less than the minimum Portuguese wage.

**Table 1. Standard Gross and Net Margin in Azorean Farms based on FADN-A Data.**

Average (euro)	Per hectare (year 2007)	Per hectare and per month (2007)
Standard Gross Margin (with subsidies)	17208.4	1434.0
Standard Gross Margin (without subsidies)	9632.2	802.7
Net margin (with subsidies)	11600.2	966.7
Net margin (without subsidies)	4024	335.3

The standard gross margin (SGM) per hectare, in 2007, was in Portugal 738€ in smaller farms and 166€ in larger farms. In Hungary, The standard gross margin per hectare was 605€ in smaller farms and 463€ in larger farms (MARTINS AND TOSSTORF, 2011). In the Azores archipelago, the value of the SGM (with and without subsidies) is higher than in Hungary or Portugal.

The standard gross margin per hectare in 2007 was for smaller farms 738€ and for larger farms 166€. In Hungary, the score of the standard gross margin per hectare was lower in smaller farm (605€) but higher in larger farm (463€) (MARTINS AND TOSSTORF, 2011). According to EU Dairy Report 2011 (EUROPEAN COMMUNITY, 2011) for milk specialized farms, the farm net income in Azores, Portugal and Hungary was respectively, 23 039€, 15 905€ and 8 020€. The net margin with coupled payments were 150€, 110€ and -19€, respectively to Azores, Portugal and Hungary. The scores of gross margin with coupled payment were 141€, 155€ and 75€ respectively to Azores, Portugal and Hungary.

### CONCLUSIONS

The subsidies are an important component of the Azorean farms, representing about a quarter of the total income. The most part of subsidies are supported by European Union, especially by PRORURAL program. In Azores, to maintain the agriculture and the farming production within a level of satisfaction it is only possible with European financial support. It is important to realize that there are not other activities in Azores archipelago that can



employ the Azorean people. Without the support of this agriculture a lot of farmers will abandon agriculture having no job alternatives, which mean that the door is open to the Azorean emigration, an usual practice in the history of Azores.

The optimization of agriculture is urgent, the farms must substitute the way of animal feeding, using more forage (pastures source), and need to find other alternatives for decreasing the cost of equipment amortisation. In this way they could increase the profit and the competitiveness of their agriculture.

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## **INFLUENCE OF INTEGRATION WITH THE EUROPEAN UNION ON ECONOMIC TRANSFORMATIONS OF FARMING IN POLAND ON THE EXAMPLE OF ŻNIN COMMUNE**

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### **ABSTRACT**

The aim of this research is to present the transformations which have been taking place in the Polish agriculture on the example of the Żnin Commune, as well as define the influence of the European Union and its support programs on the range and pace of these changes. The survey was carried out in 2010, involving 172 owners of agricultural farms. A method of a survey was used. The results have been analyzed statistically. The surveys revealed that in the examined parish there were favorable conditions for the development of agriculture. This is largely connected with the increasing popularity of funds supporting transformations, the development of rural areas and active involvement of farmers in gaining this kind of support. In the future the transformation processes of farms will depend mainly on the course of changes in the European Union Common Agricultural Policy which is the main factor affecting the shape of this sector of economy. The course of change of rural areas and the future of farmers and their farms will depend on the form and quantity of support directed to rural areas. An important factor which affects shaping the transformation process will also be the awareness level of farm owners and their access to information.

**Keywords:** economic transformations of rural areas, influence of the integration with the EU, financial support for agriculture and rural areas development, the Common Rural Policy

### **INTRODUCTION**

In terms of economic and social sciences, transformations and evolution in the XIX and XX centuries can be expressed using the concept of commercialization of farming. Farming has gone a long way from natural to market farming. This process covered most of countries, but the pace of its development was diversified. In its evolution farming has gone through three stages: self-sufficient, diversified and specialist farming (OTOLŃSKI, WIELICKI, 2003). The changes which have taken place in Poland during last several years have had a large influence on the way of farming which in the initial stage went through a serious crisis. However, gradually the situation of farming has been improving. Thanks to having overcome difficulties connected with the transformation period and subsequent closer cooperation with the EU in the pre-accession period and integration with the Common Agricultural Policy after the accession, the Polish farming has become more competitive and its economic situation has significantly improved (BAŃSKI, 2007). Transformations that are taking place in agriculture aim at developing this industry and this can be a factor affecting growth of the rural population's income. Change in the agrarian structure, ownership, as well as the level and role of the state interference with the agricultural market, are the main factors affecting the development of farming which in turn has a large influence on fighting unemployment, propagating innovativeness and new technologies which improve work. Thus, a multi-functional growth of farming is essential for transformation of the Polish agriculture. The general tendencies of transformations, specified above, will be enhanced or impaired by different factors including the economic



situation of the country, its policy and the policy of the European Union in the field of agriculture.

## **MATERIAL AND METHOD**

The considered survey was carried out in 2010, on the territory of Żnin Commune which is situated in the north-west part of the Kujawsko-Pomorskie Province and is of rural-urban character, where farming plays a significant part. Its area is 251.55km<sup>2</sup>, including 81% of arable lands and 6% of forests. The quality indicator of agricultural production space is relatively high, being 82.6 points (for 100 possible). Agricultural production is realized on arable lands with a small share of grassland and orchards. The structure of arable land is as follows: arable land – 18805 (93.15%), grassland 1247.5 ha (6.18%), orchards -135.5 ha (0.67%).

A survey method was used in the research. The research tool was an earlier prepared questionnaire form. This questionnaire was based on a five-step Likert's scale which is often recommended in social research methodology and enables to obtain answers concerning the acceptance degree of the examined phenomenon. This scale consists of a cafeteria with five replies arranged in an alphabetical order, beginning from the level of absolute acceptance to absolute rejection. The obtained data was elaborated with the use of methods of statistical-mathematical and substantial analysis in order to account for dependencies of the studied variables and establish relations of the considered phenomena. A standard descriptive analysis has been employed, and the calculation sheet Microsoft Excel has been used for the analysis of the research material. The gathered data has been elaborated in the form of tables and charts.

172 respondents, agricultural farm owners, situated on the territory of Żnin Parish, took part in the survey. The size of the sample group was determined from the formula:

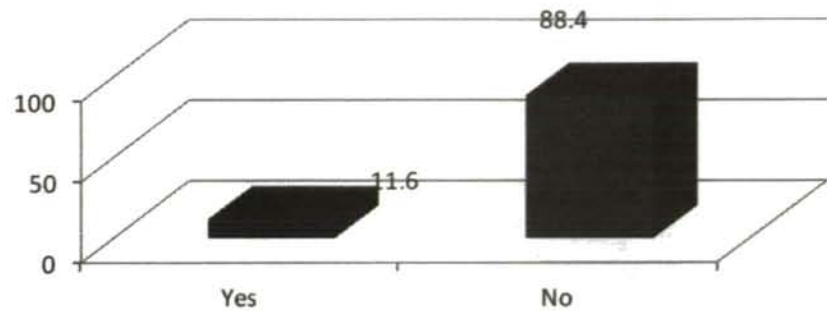
$$N_b = \frac{N}{1 + \frac{4d^2(N-1)}{z_\alpha^2}}$$

where: N-number of population; d-admissible error of estimation;  $z_\alpha$ -1.64 (for  $\alpha=0.10$ ). For this sample group size the obtained results were analyzed with probability 95%, and measurement error 6%.

## **RESULTS**

As early as before accession of Poland to the European Union, the Polish farmers could take advantage of financial support provided by SAPARD (Special Accession Program for Agricultural and Rural Development), which was an instrument of the pre-accession support for agriculture and development of rural areas directed to ten countries applying for membership in the EU. However, according to T.G. Grosse [2005,s.2] it was mainly large companies and farms which benefited from this program, whereas it was not well taken advantage of by small farms and it did not have a big influence on their consolidation.

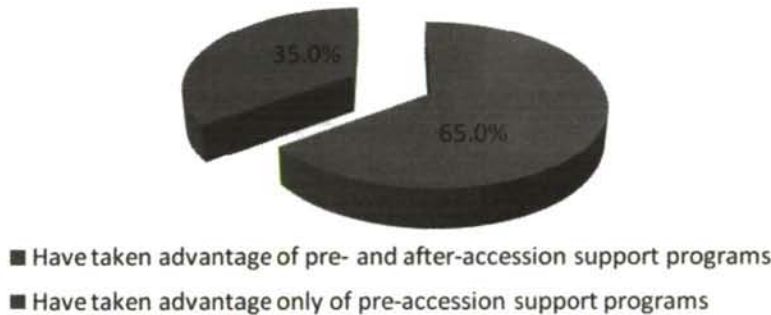
A great majority of the surveyed population did not take advantage of the pre-accession financial support programs offered by the European Union (88.4%). Only 11.6% of respondents admitted having accepted SAPARD support (fig.1). Those were mainly owners of farms with the area from 20.1 to 50 ha (90.0%). Minority of the farmers, that is 10.0% who had taken advantage of pre-accession programs, owned farms larger than 50 ha. The examined group of farmers did not include persons who had used the SAPARD program and owned farms smaller than 20.1 ha.



**Figure 1. Use of pre-accession SAPARD program by respondents in %**

Source: own research

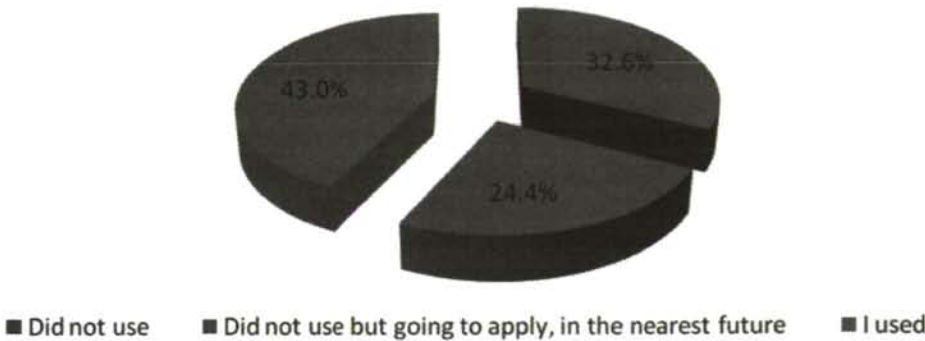
Farmers who had already used the EU support funds for the development of their farms were found to be keen on using SAPARD program again. Great majority of the surveyed farmers who had taken advantage of the SAPARD program declared that they had taken part in other European Union programs (65.0%). Only 35.0% who had benefited from SAPARD did not take part in any past-accession support scheme (fig.2).



**Figure 2. Declarations on the use of post-accession support programs made by farmers who had taken advantage of pre-accession support within SAPARD**

Source: own research

No wonder, the surveyed farmers were more interested in past-accession support programs than in SAPARD. About four times more farmers declared having accepted help from such programs as Plan of Rural Areas Development 2004-2006 (PRAD 2004-2006) and/or Program of Rural Areas Development 2007-2013 (PRAD 2007-2013). In the examined group of farmers 57.0% did not use past-accession financial programs from the EU funds, though there were persons (24.4%) who were not going to benefit from support programs in the nearest future (fig.3).

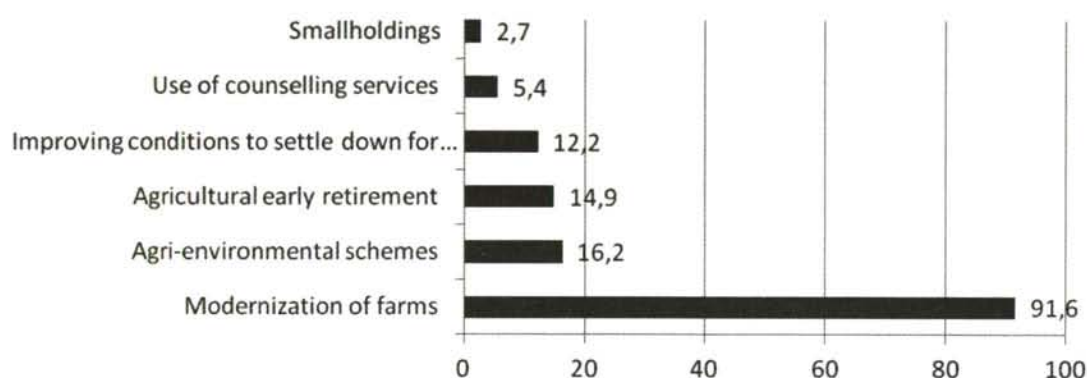


**Figure 3. Declarations of respondents on using past-accession programs (PRAD 2004-2006 and/or PRAD 2007-2013)**

Source: own research



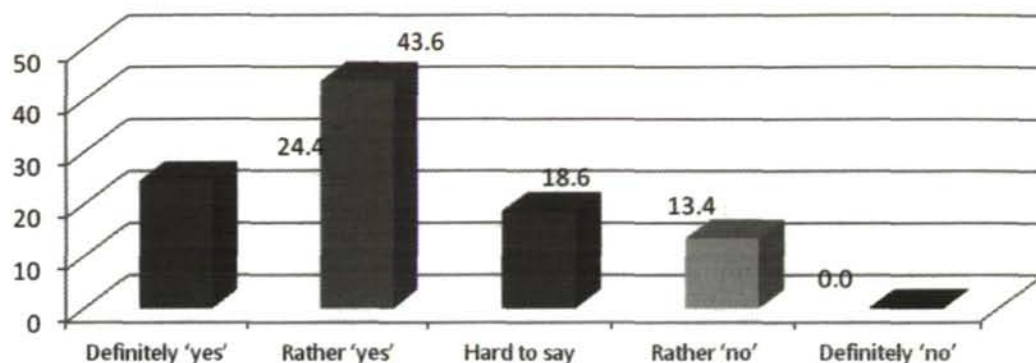
The most frequently declared action taken by the respondents within the support programs after the integration with the European Union was modernization of farms (91.9%). Next, there were agri-environmental schemes (16.2%) and agricultural early retirement (14.9%). 12.2% of respondents benefited from a bonus for young farmers within the program supporting young farmers in setting up their businesses. Few interviewees, not more than 5.4% of those who had used past-accession programs, chose to apply for funds connected with counseling services for farmers and forest owners. In the surveyed group there were few farms benefiting from the EU funds within the so called smallholdings (2.7%). The percentage sum exceeds 100.00% as the respondents could mark all activities they were involved in (fig.4).



**Figure 4 Actions taken by respondents (%) within PRAD 2004-2006 and/or PRAD 2007-2013**

Source: own research

Another form of support available to the Polish farmers after the integration with the European Union are direct subsidies which aim at compensating reduction of minimal and prevention prices for agricultural products on the European Union market. In the Accession Treaty it is stated that Poland is allowed to use a simplified system of direct subsidies. Today these subsidies have become an important factor stabilizing farmers' incomes making it possible to improve the economic situation of farms without raising prices of agricultural products, with constantly growing production costs. The EU direct subsidies, co-financed by the national budget, provide the customers with cheap food and make the EU products competitive on international markets. According to the Agency for Restructuring and Modernisation of Agriculture (ARMA), the direct subsidies are used by 1.4 mln of the Polish farmers [<http://www.arimr.gov.pl/doplatty-bezposrednie.html>]. All the interviewed farmers declared having benefited from direct subsidies. They replied positively to the question concerning whether direct subsidies had improved the economic situation of their farms, most answers 43.6% of the respondents were 'rather yes'. The answer 'definitely yes' was declared by 24.4% of farmers and 18.6% had no opinion on this subject. The analyzed group also included persons who were of the opinion that the subsidies did not have much influence on the financial condition of their farms (13.4%). However, no respondents expressed an opinion that the direct subsidies had no influence on the economic situation of their farms (fig.5).



**Figure 5. Declarations of respondents (%) on the subject of a positive impact of received subsidies on the financial condition of their farms**

Source: own research

## CONCLUSIONS

The results of the presented survey predict good conditions for the development of farming in this commune due to the right course of transformations initiated after the integration with the European Union and providing the possibility of taking advantage of support funds directed to rural areas. Such programs as SAPARD, or PROW (Program of Rural Areas Development) 2004-2006 and PROW 2007-2006, undoubtedly have an influence on the changing economic-social and technical situation in agriculture. Thanks to this the scale of investments is increasing and work is becoming simpler and more efficient. For most of the respondents, the possibility of modernization of their farms was crucial to decide whether or not to apply for the European Union funds co-financed from the national budget. Other forms of support co-financed from the EU means, that is, direct subsidies were used on a regular basis in the studied farms. According to the interviewed farmers, they have also significantly contributed to the improvement in the farms income situation. Thanks to them it was possible to compensate partly the low prices of agricultural products, with increasing prices of production means. The direct subsidies significantly affected the increase in the price of the land. Land in Poland became attractive not only for farmers but also for entrepreneurs and investors as a good investment for the future. With the land getting more and more expensive the popularity of leasing has also increased. In the future, in consequence of further transformations, the conditions for farming may also significantly improve. Transformation processes in farming can be a sign of positive tendencies both for the change of intellectual resource capital structure and an increase in the area and modernization of the farms technical equipment. The European Union is a factor which has the largest influence on the shape of agriculture. The form of support will be crucial for this economy sector, the course of development and the future of farmers and their farms. Also the level of farmers' awareness will play a very important role in shaping the transformation processes. New Common Agricultural Policy will be essential not only for farmers but also for each inhabitant of the Union. It aims at increasing the share of ecological and healthy food, simultaneously following the rules of sustainable development of the natural environment, providing farmers with its resources and being actively shaped by them. However, the size of rural population's incomes might change



significantly, with accompanying diversification of agricultural farms. According to predictions of many experts in the field of farming economics, the importance of commercial farms will increase, whereas small production farms will weaken. Part of them will limit to 'self-supply' and will gradually be eliminated from the market. The remaining ones will have a multi-functional character and they will become family agricultural-production firms or agricultural-service ones (ADAMOWICZ, 2005; BRODZIŃSKI, LEWCZUK, 2001; WIATRAK, 2001; WILKIN, BUDZIUCH-SZUKAŁA, SALONI, 2005; ZAWISZA, ADAMCZEWSKA, 2009).

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**THE EFFECTIVENESS OF SOME CHEMICALS AGAINST POSTHARVEST DISEASES CAUSED BY *PENICILLIUM DIGITATUM*, *PHYTOPHTHORA CITROPHTHORA* AND *GEOTRICHUM CANDIDUM* ON ORANGE**

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**ABSTRACT**

The effectiveness of the mixture of Xedabio (1.5%) + KPhos (0.5%) + Xedathane (0.25%) for postharvest control of green mold (*Penicillium digitatum*), brown rot (*Phytophthora citrophthora*) and sour rot (*Geotrichum candidum*) infections on orange c.v. "Jaffa" was investigated. Oranges stored at 18-20 °C for 24 hours were inoculated with spore suspension (10<sup>6</sup> spores/ml) after injured on the equatorial area. The chemicals were applied with water at 21±1°C and 46±1°C, as two groups. Inoculated fruits were incubated at 9-10 °C in a storage controlled by TFA Datalogger during 15 days. The trial was set up according to the randomized complete block design with three replications. The diameters of symptoms on each fruits were measured with electronic compass everyday.

The interaction between the treatment conditions and storage time were significant at 1% significance level. The mixture of Xedabio + KPhos + Xedathane applied with water at 46±1°C was found more effective than the same mixture used with water at 21±1°C.

As a result, the mixture of Xedabio + KPhos + Xedathane in different water temperatures could be used against some postharvest diseases on orange.

**Keywords:** Orange, post-harvest disease, Xedabio, KPhos, Xedathane.

**INTRODUCTION**

The citrus production of the world is about 123,755,750 tons, and 56% of this is orange. According to 2010 data, there were produced 1710.50 tons of orange in the field of 53,236 hectares in Turkey (FAO, 2010). Among the mediterranean countries, Turkey takes first place with this orange production (TÜİK, 2009).

The post-harvest fungal diseases can cause significant losses of about 20% to 50% of the yield of fresh vegetables and fruits (KLEIN AND LURIE, 1991). They cause serious problems to the harvested citrus fruits during handling, transportation, exportation and the storage process (HOW, 1991; WILSON ET AL., 1994). The post-harvest fungal spoilage was mostly due to green and blue molds (*Penicillium digitatum* and *P. italicum*) and sour rot (*Geotrichum candidum*) (BALI ET AL., 2008; EL-MOUGY ET AL., 2012). The use of synthetic fungicides gave satisfactory control against mold infection. But fungicidal residues can have harmful effect on people and environment (ECKERT AND OGAWA, 1988; WILSON ET AL. 1994). In addition, the excessive and improper use of fungicides could lead to the development of fungi races being resistant against the applied fungicides. Therefore, alternative chemicals are needed for the postharvest management of orange fruits. In this respect, nowadays the researches on microbial biocontrol agents and alternative chemicals against postharvest diseases have been increasing (JANISIEWICZ AND KORSTEN, 2002; HUANG ET AL., 1995; BULL ET AL., 2006).



The main pupose of this study is to investigate the effectiveness of an alternative chemical mixture against post-harvest diseases (*Penicillium digitatum*, *Phytophthora citrophthora* and *Geotrichum candidum*) on orange fruits.

## MATERIAL AND METHOD

### Plant material

Orange fruits (*Citrus sinensis* Lin. c.v. "Jaffa") were obtained from a private orchard in Köyceğiz town (Muğla province). Harvested fruits were transported by a vehicle (fruco) to Çanakkale on the same day. The fruits were stored at 18 to 20 °C for 24 hours until application.

### Fungi and inoculum

*Penicillium digitatum*, *P. digitatum* and *Geotrichum candidum* were isolated from infected orange fruits. The isolates were grown on PDA medium (39 g potatoes dextrose agar / 1 liter sterile distilled water) at 25 °C for 7 days. At the end of the incubation sterile water and Tween were added into Petri dishes and conidia were passed into the water by rubbing with glass rod. Fungal suspension was filtered through two layers fine muslin to remove the mycelium and other fragments. Spore suspension was then counted with a haemocytometer and adjusted to  $10^6$  spores / ml. The orange fruits of "Jaffa" variety were wounded by the tip of sterile micropipette in the equatorial areas of fruit peels. They were inoculated with 60 µl spore suspension from their wounded side and left for incubation at room temperature (21 °C-22 °C) during 12 hours.

### Treatments and storage conditions

After incubation, orange fruits were separated into two groups and treated with the mixture of Xedabio (1.5%) + KPhos (0.5%) + Xedathane (0.25%) in water at two differend tempratures (21±1 °C and 46±1 °C). Control fruits were only treated with warm water. Then, all of the fruits were dried for one hour naturally. Orange fruits were stored at 9–10 °C and 85-90% RH conditions after inoculation in a storage controlled by TFA Datalogger for 15 days. The daily improvement of the infected area was measured by digital calipers.

### Statistical analysis

The experiment was arranged in randomized complete design with 3 replications. 10 fruits were used for each replication. Analysis of variance was conducted on the efficacy of the chemical mixture by using the Tukey Method and means were compared by using LSD Range Test at  $p<0.01$  level. Effectiveness of the chemical mixture on disease was calculated by Abbott formulation.

$$\text{Corrected \%} = \left( 1 - \frac{\text{infected area in T after treatment}}{\text{infected area in Co treatment}} \right) \times 100$$

T= Treated Co= Control

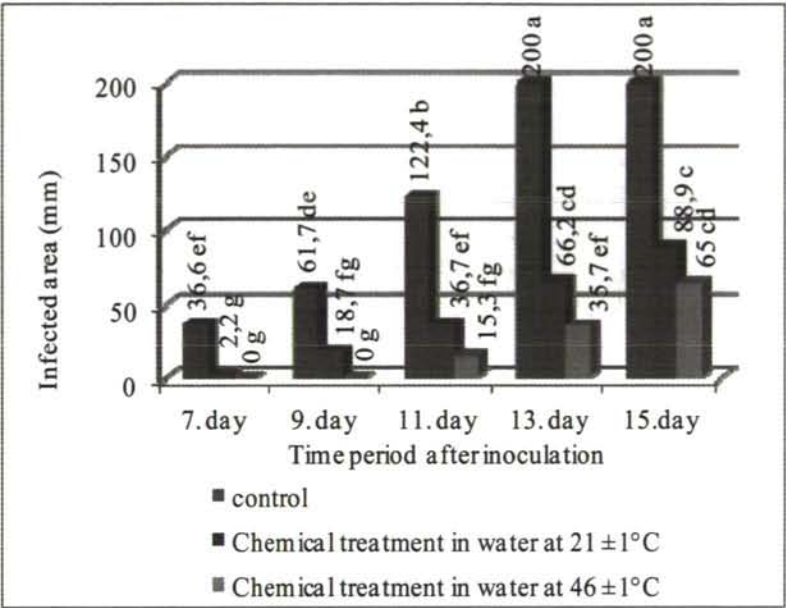
## RESULTS

Orange fruits inoculated with *Penicillium digitatum*, *Phytophthora citrophthora*, *Geotrichum candidum* were treated by the combination of Xedabio (1.5%) + KPhos (0.5%) + Xedathane (0.25%) in dipping water with two different temperatures (21±1°C and 46 ±1°C). The combined treatment at both water temperatures affected three mold types.

### Green mold (*Penicillium digitatum*)

Control fruits inoculated with *Penicillium digitatum* were all infected on the 9<sup>th</sup> day. However, 22% of fruits treated by the combination with dipping water at 21±1°C were infected. Furthermore, infection in fruits treated by the combination with dipping water at

46 ±1°C was 18%. Moreover, 47% of fruits treated at both 21±1°C and 46 ±1°C were infected on the 15<sup>th</sup> day after inoculation. Infected area increased on all fruits. Besides, this increase was highly determined on the control fruits (Fig. 1).



**Figure 1. Improvement of green mold on orange storage as infected area**

Infected area increased rapidly after the 7<sup>th</sup> day of inoculation. However, this increase was slower in treated fruits (Fig. 1). No significant difference was fixed between fruits treated at different water temperatures. However, significant difference between treated and untreated fruits was determined ( $p=0,01$ ). Furthermore, significant difference between treatment temperatures was fixed on the 13<sup>th</sup> day after inoculation (Fig. 1). The combination of three chemicals prevented the growth of green mold on orange fruits during 15 days storage (Table 1). The level of effect increased with dipping water temperature.

**Table 1. Effects of chemical mixture on green mold caused by *P. digitatum* on orange fruits (%)**

Time period after inoculation	Chemical treatment in water at 21 ±1°C	Chemical treatment in water at 46 ±1°C
7. day	94.0 a	100 a
9. day	69.7 ab	100 a
11. day	70.0 bc	87.5 ab
13. day	66.9 de	82.2 bc
15. day	55.6 e	67.5 de

**Brown rot (*Phytophthora citrophthora*)**

Control fruits inoculated with *Phytophthora citrophthora* were all infected on the 9<sup>th</sup> day. However, 11% of the fruits treated at 21±1 °C and 7% of the fruits treated at 46±1 °C were infected in the same period. Furthermore, 51% of the fruits treated at 21±1 °C and 37% of the fruits treated at 46±1°C were infected on the 15<sup>th</sup> day after inoculation. The improvement of brown mold was at highest level in control fruits compared to treated fruits. The difference was found to be significant ( $p<0.01$ ) (Fig. 2).



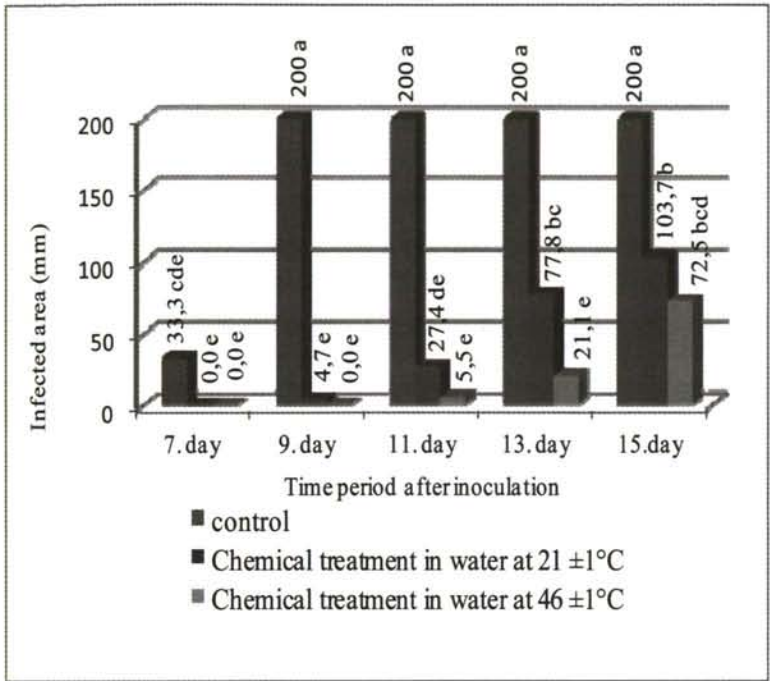


Figure 2. Improvement of brown rot on orange storage as infected area

The maximum level of infected area in control fruits was materialized on the 9<sup>th</sup> day. The area on the treated fruits was significantly lower ( $p<0.01$ ). Moreover, minimum infected area was found on fruits treated at  $46\pm1^{\circ}\text{C}$  (Fig. 2). Combined treatments were found to be protective against brown rot during the whole storage (Table 2).

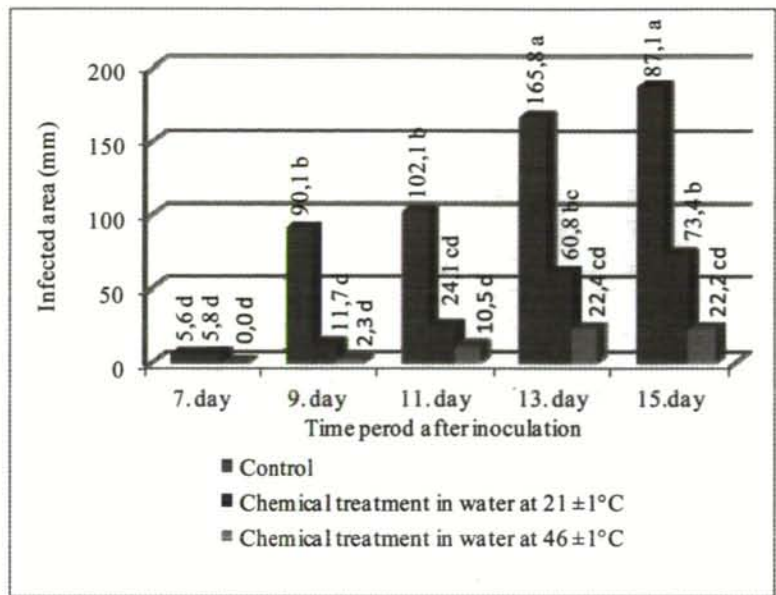
Table 2. Effects of chemical mixture on brown rot caused by *P. citrophthora* on orange fruits

Time period after inoculation	Chemical treatment in water at 21 ± 1°C	Chemical treatment in water at 46 ± 1°C
7. day	100 a	100 a
9. day	97.6 a	100 a
11. day	86.3 ab	97.2 a
13. day	61.1 cd	89.4 a
15. day	48.2 d	63.8 abc

The difference of effects between treatment temperatures became visible by the 13<sup>th</sup> day after inoculation. Thus, the treatment at  $46\pm1^{\circ}\text{C}$  was found to be more effective. (Table 2).

**Sour rot (*Geotrichum candidum*)**

The infection rate of control fruits achieved 81% on the 9<sup>th</sup> day after inoculation. Besides all fruits became infected with *G. candidum* after 13 days. However the spread of infection reduced with treatments. Thus, the rate of infection was 19% on fruits treated at  $21\pm1^{\circ}\text{C}$  on the 9<sup>th</sup> day and increased to 44% till the 15<sup>th</sup> day after inoculation. Similar and more visible results were determined on the fruits treated at  $46\pm1^{\circ}\text{C}$ . In this context the rate of infection was 7% on the 9<sup>th</sup> day and 11% on the 15<sup>th</sup> day. The symptoms became visible on the 7<sup>th</sup> day after inoculation. The infected area was determined at higher levels significant in control fruits ( $p<0.01$ ) (Fig. 3).



**Figure 3. Improvement of sour rot on orange storage as infected area**

The increase of the infected area in control fruits was similar to that of the treated fruits until the 7<sup>th</sup> day. However, a significant difference occurred by the 9<sup>th</sup> day ( $p < 0.01$ ). Furthermore, no significant difference was determined between treatment temperatures until 13 days. Besides, significant difference in infected area between treatment temperatures was determined on the 15<sup>th</sup> day ( $p < 0.01$ ) (Fig. 3).

Combined treatment affected the growth of sour rot during 15 days of storage (Table 3). Furthermore, significant difference between treatment temperatures occurred 15 days after inoculation ( $p < 0.01$ ). The most effective treatment temperature was found as  $46 \pm 1$  °C.

**Table 3. Effects of chemical mixture on sour rot caused by *Geotrichum candidum* on orange fruits (%)**

Time period after inoculation	Chemical treatment in water at 21 ± 1°C	Chemical treatment in water at 46 ± 1°C
7. day	0 d	100 a
9. day	87.0 a	97.4 a
11. day	76.4 ab	89.7 a
13. day	63.3 bc	86.5 ab
15. day	60.8 c	88.1 ab

## DISCUSSION AND CONCLUSION

Pathological disorders cause significant postharvest losses (KLEIN AND LURIE, 1991). Synthetical fungicides are used to prevent these losses, however residues are the main problems including these treatments (ECKERT AND OGAWA, 1988; WILSON ET AL., 1994). Thus, alternative chemicals and usages are found against postharvest pathological disorders without residues.

*Penicillium digitatum*, *Phytophthora citrophthora* and *Geotrichum candidum* are the main fungi of orange in the postharvest period.

In this research, the semi *in vivo* effect of Xedabio, KPhos and Xedathane combination was determined. Xedabio including clove oil is a natural compound used against postharvest molds in citrus, stone fruits and pome fruits. Besides, it can be combined with synthetic



fungicides. Xedathane includes prymethanil and was found effective againsts grey mold in many fruit and vegetable species (HINTON AND INGRAM, 2005). In addition, potassium phosphite (KPhos) was found effective againsts green mold (*P. digitatum*), blue mold (*P. italicum*), phomopsis and *Alternaria citri*, respectively.

In this research, the combination of Xedabio, KPhos and Xedathane at different water dipping temperatures such as  $21 \pm 1$  °C and  $46 \pm 1$  °C prevented green mold, brown rot and sour rot significantly during 15 days of storage. The effect of combined treatment increase with hot water treatment, however combination of Xedabio, KPhos and Xedathane prevented these pathological disorders even at  $21 \pm 1$ °C.

According to the results, combined treatment of Xedabio (1.5%) + KPhos (0.5%) + Xedathane (0.25%) prevented and reduced the spread of *Penicillium digitatum*, *Phytophthora citrophthora* and *Geotrichum candidum* in orange fruits without residue problem. Thus, the treatment including this combination can be used in fruit industry.

### ACKNOWLEDGEMENTS

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## TESTING THE STABILITY OF GRAIN YIELD AND BREAD-MAKING QUALITY OF WHEAT VARIETIES IN TWO DIFFERENT YEARS

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### ABSTRACT

25 winter-type bread wheat genotypes were evaluated in two consecutive years (2010 and 2011) in the nursery of Cereal Research Non-Profit Company (CRNPC) to test the stability of grain yield and quality traits of CRNPC-bred varieties. In spite of the earlier trends the extremely wet 2010 year's grain yield became significantly lower and bread-making quality proved to be poorer than in the dry 2011 year. The most significant reasons of those found to be the very strong disease (mostly leaf rust and *Fusarium*) infection pressure in the highly precipitated 2010 year. Other, minor reasons were water logging stress, and harvest deficits due to the remarkable lodging of wheat. Stability of grain yield and different quality traits (wet gluten content, gluten stability, kernel hardness, farinograph water absorption, farinograph value, *Zeleny*-value, falling number) were evaluated by regression calculations to test the varieties' adaptability to the different year effects. In case of yield, a wide variation was found in stability of grain output. In cases of quality traits, the most sensitive traits were falling number, farinograph water absorption and developing time of dough.

**Keywords:** Stability of grain yield, stability of bread making quality, leaf rust, fusarium, effect of year

### INTRODUCTION

Stability of grain yield and technological (or bread making) quality are crucial points in wheat production. Growing wheat under different conditions makes difficult to produce a predictable yield and quality level. Increasing occurrence of weather anomalies and extremities also extends the necessity for new genotypes with higher adaptation capacity. Different seasons with diverse water supply or disease infestation pressure may change the order of genotypes score in grain yield and quality significantly (CSEUZ ET AL., 2006). Breeders try to answer these challenges by multi-location testing of advanced lines to find the most adaptable ones among the ones with high yield potential and technological quality (CSEUZ ET AL., 2008, FÓNAD ET AL., 2007). In this paper we tried to answer the questions that how can wet years give lower yield and quality, than a dry one, and does our breeding material have ample genetic variation in adaptability.

### MATERIAL AND METHOD

25 genotypes (*Table 1*) of winter type bread wheat (*Triticum aestivum* L.) were tested in two consecutive years (2010 and 2011) in the nursery of Cereal Research Non-Profit Co., Szeged – Kecskés (46° 14' 04.50''N; 20° 05' 09.91''E). Growing conditions modelled the conventional wheat technology (best practice without fungicide treatments) which is followed by most farmers in the country, adding 70+70+70 kg of (NPK) basic fertilizer and 120 kg N fertilizer extra at spring time, and applying plant protection treatments



against pests and weeds. For the disease resistance evaluation not any fungicide treatment was applied. In the four replicated field trials the plot sizes were 6.5 m<sup>2</sup> in both years. The seeds of all genotypes were taken from the previous year's variety maintenance plots. To compare not only the yield performance but quality level of the varieties in different years, besides the application of micro testing methods on all the samples (H.I. studies, diameter, thousand kernel mass (TKM), NIR wet gluten, protein content, falling number, Zeleny-tests) we determined flour yield, wet gluten content, gluten elasticity, and farinograph value. In both years we evaluated two times the infestation scores of the two mostly current fungal diseases leaf rust (*Puccinia recondita*) and *Fusarium* head blight (*Fusarium graminearum*) The presence of other wheat diseases like powdery mildew (*Blumeria graminis*) or leaf spots (*Drechslera* and *Septoria* species) did not hit a level of momentous epidemics. After harvest we performed correlation calculations between the disease scores and yield losses and mostly affected quality deficit data. To find the best genotypes in stability of grain yield and quality characters, we made regression calculations among the certain traits' two years values.

Table 1. The most important traits of varieties and variety candidates in the survey

The most important traits of varieties and variety candidates in the survey									
Number	Code of genotype	Year of registration	Precocity	Quality class	Type of head	Resistance to			
						Blumeria g. (E.g)	Puccinia recondita	Puccinia graminis	Fusarium sps.
1.	GK 12.93	1996	early	milling I	awnless				4,5
2.	GK 03.98	2001	late	milling I	awnless				2,3
3.	GK 34.02	2005	early	improver	awned				3,6
4.	GK 38.02	2005	early	milling I	awnless				2,6
5.	GK 18.03	2006	early	milling I	awnless				2,1
6.	GK 39.06	2009	early	milling I	awned				1,8
7.	GK 16.07	2010	early	milling I	awned				4,4
8.	GK 21.07	2010	early	milling I	awned				2,4
9.	GK 28.07	2010	early	milling I	awnless				1,6
10.	GK 38.07	2010	early	milling I	awnless				2,8
11.	GK 45.06	2010	early	milling I	awnless				3,3
12.	GK 10.08	2011	early	milling I	awnless				3,8
13.	GK 03.09	candidate	early	milling I	awnless				2
14.	GK 04.09	candidate	early	milling I	awnless				2,3
15.	GK 06.09	candidate	early	milling I	awnless				3,3
16.	GK 09.09	candidate	early	improver	awnless				1,7
17.	GK 02.10	candidate	early	milling I	awnless				1
18.	GK 04.10	candidate	early	milling I	awned				2
19.	GK 14.10	candidate	medium	milling I	awnless				2,5
20.	GK 17.10	candidate	medium	milling I	awnless				4
21.	GK 20.10	candidate	medium	milling I	awnless				1,5
22.	GK 41.10	candidate	medium	milling I	awnless				5
23.	GK 42.10	candidate	early	milling I	awnless				3,5
24.	GK 46.10	candidate	early	milling I	awnless				1,5
25.	GK 47.10	candidate	medium	milling I	awnless				4,5

excellent

medium

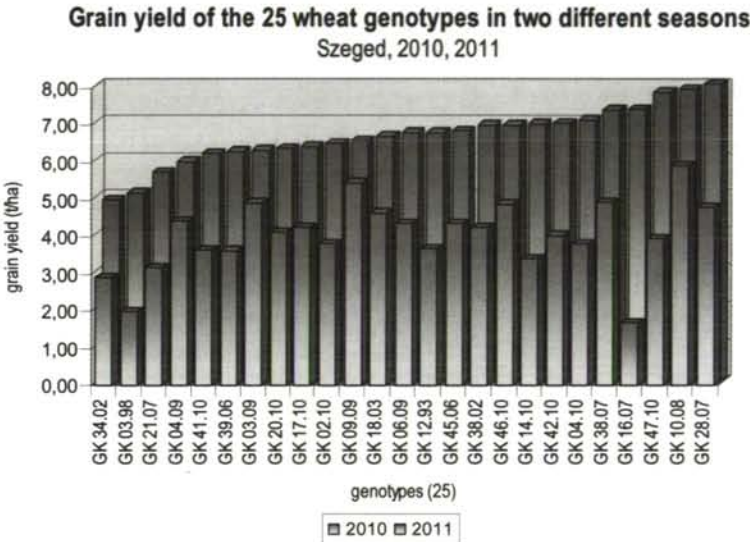
good

poor

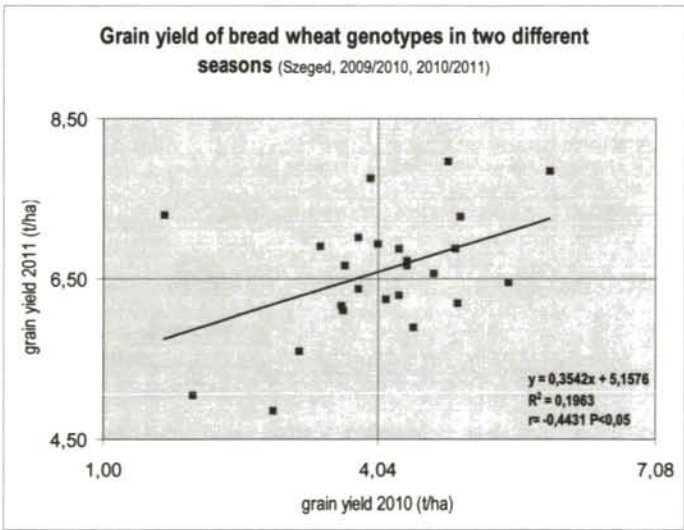
RESULTS

Grain yield (Figure 1) and grain weight (thousand kernel mass) worked out on a significantly lower level due to the biotic and abiotic stresses in the year 2010. The average grain yield of the 25 wheat genotypes was 4.04 t/ha in the year 2010, while it was 6.59 t/ha in 2011. Not at the same extent but similarly the average thousand kernel mass also was significantly lower in 2010 (31.4 vs. 37.6 g). We have found significant correlation between the two years' grain yield data (Figure 2). It shows that most of the tested genotypes can cope with the different environmental factors. All the genotypes present in the upper right part in Figure 2 have the highest yielding ability and the best adaptability

(stability of yields) since they performed over the average in both years. The best grain yield stability was found in the case of varieties GK10.08 (GK Futár), GK28.07 (GK Körös), GK 38.07 (GK Vitorlás) and GK 38.02 (GK Csillag). In the lower right part are the lower yield capacity wheat genotypes with good yield stability under any conditions (extensive wheat varieties). In the left upper corner are the „super intensive” cultivars which have no good adaptability and perform well only under optimal conditions. One part of the left lower corner varieties have a high tolerance to abiotic stresses but yield on a low level, but the other ones have a quite good yielding ability with a very poor adaptability. All of the four categories contain registered varieties and new candidates as well.



**Figure 1. Grain yield of the 25 wheat genotypes under two different weather conditions**

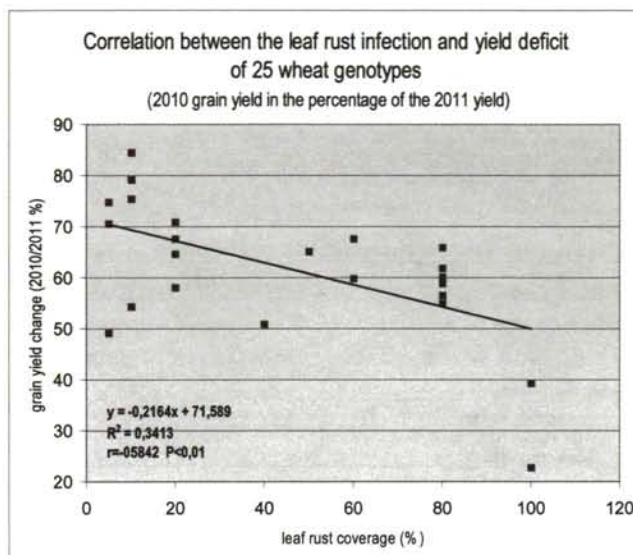


**Figure 2. Correlation between grain yield of 25 wheat genotypes in two diverse growing seasons**

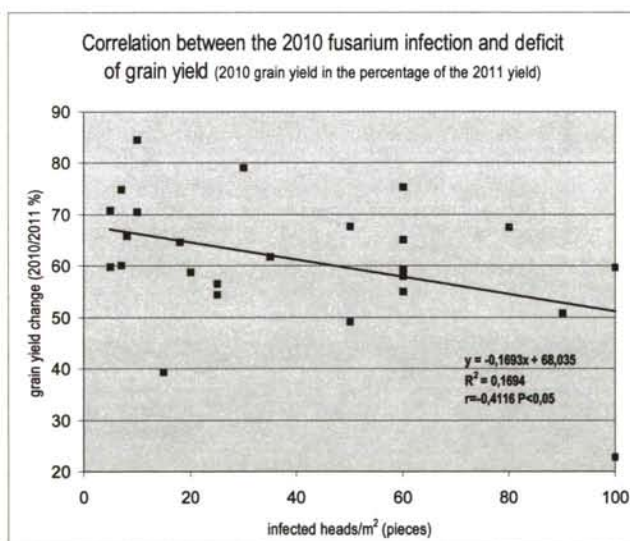
According to our regression calculations we found a significant, moderately close correlation ( $r = -0.5842$ ;  $r = -0.4116$ ) between the change of grain yield and the genotype's leaf rust (*Puccinia recondita*) (Figure 3) and head blight (*Fusarium graminearum*)



(Figure 4) infection data in 2010. Since these two types of diseases were dominant in our nursery yield loss presumably happened due to the direct and indirect effects of these species.



**Figure 3. Correlation between leaf rust infection and yield deficit of 25 wheat genotypes**



**Figure 4. Correlation between fusarium infection and yield deficit of 25 wheat genotypes**

In bread making quality we made a comparison between the two years' data, and we found that the most unstable traits were Hagberg-falling number (21% decrease), dough forming time (41.1% shorter in 2010), Farinograph-value (24.7% lower in 2010) and ICC stability which was 46.4% poorer in the wet year. We found significant correlation between the two different years' farinograph value and Hagberg-falling number data (Figure 5 and 6). This means that in both traits we have genotypes with a good stability in these characters. Most of the quality traits of variety candidates GK 41.10, GK 42.10 and varieties GK 10.08 (GK

Futár), GK 21.07 (GK Rozi), GK 34.02 (GK Békés) and GK 38.02 (GK Csillag) proved to be more stabile against biotic an abiotic stresses, than the mean of the genotypes tested. On the other hand, no correlation was found between the 2010 year disease infection (leaf rust covering and fusarium-infected spikes per square meter) and the deficit in quality traits (Table 2).

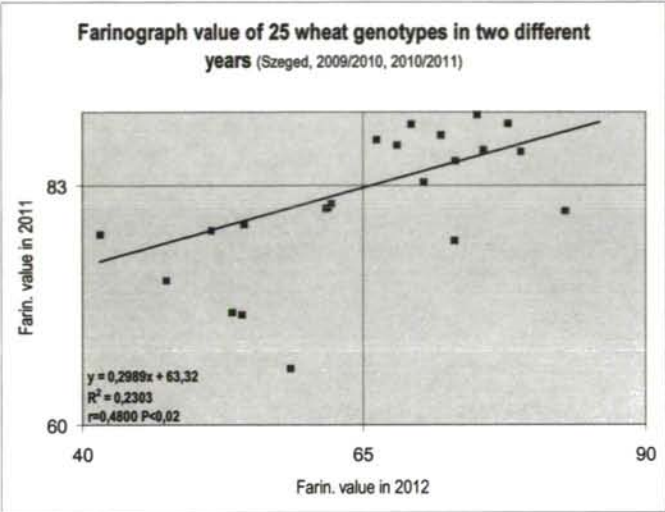


Figure 5. Stability of bread making quality: correlation between the farinograph values of 25 wheat genotypes in two different years

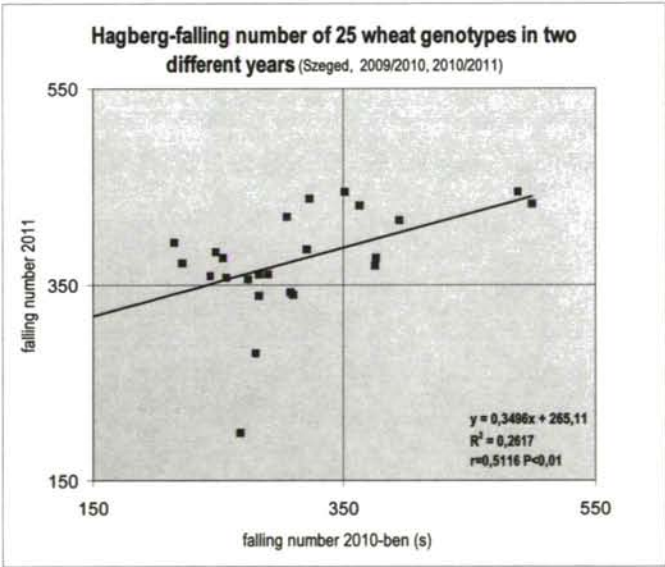


Figure 6. Stability of bread making quality: correlation between the falling number data of 25 wheat genotypes in two different years



**Table 2. Correlation between the 2010 fungal disease infection and the change in the most unstable quality traits of 25 wheat genotypes**

	Elasticity of gluten (mm)	Forming time of dough	Farinograph value.	ICC stability	Leaf rust coverage	Fusarium head no.
Elasticity of gluten (mm)	1					
Forming time of dough	-0,0425	1				
Farinograph value.	0,1087	0,3696	1			
ICC stability	0,1573	0,1419	0,8983	1,0000		
Leaf rust coverage 2010	<b>-0,0897</b>	<b>-0,1475</b>	<b>0,1169</b>	<b>0,0060</b>	1	
Fusarium head no.2010	<b>-0,0133</b>	<b>0,2128</b>	<b>0,0986</b>	<b>-0,0121</b>	0,2236	1

### CONCLUSIONS

Wheat year 2009/2010 was unfavorable for winter wheat in the most part of Hungary because of the tremendous amount of precipitation and the phytopathology status evolved. In spite of that the next year was an extremely dry one, significant drought stress could not develop in wheat year 2010/2011 due to the soils' high moisture content from the previous year.

Mostly the diseases, namely leaf rust (*Puccinia recondita*) and fusarium head blight (*Fusarium graminearum* and *Fusarium culmorum*) are responsible for the low grain yields of 2010. Minor reasons are water logging stress for the root system and harvest losses due to the remarkable lodgings of wheat. According to our survey we could not prove that deficits in quality characters happened directly to disease infections. It seems that the reasons of quality deficits are complex, and are due to biotic and abiotic environmental stresses as well.

Both in stability of grain yield and stability of bread making quality we found a significant variation among the 25 CRNPC-bred genotypes tested. The best grain yield stability was found in case of GK Futár, GK Körös, GK Vitorlás and GK Csillag, while in the stability of quality best performers were GK 42.10, GK 41.10, GK Csillag and GK Futár.

### ACKNOWLEDGEMENTS

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## LANDSCAPE FACTORS INFLUENCING ROE DEER ROADKILL FREQUENCIES ON THE M3 HIGHWAY OF HUNGARY

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### ABSTRACT

Roe deer (*Capreolus capreolus*) is one of the most dangerous one for traveller's safety amongst the regularly occurring, conflicting wildlife species on the Hungarian highways. Severity of roe deer collisions might be minimized by manipulating its influencing factors, but these factors are firstly to identify and recognize. In this study we investigated the landscape features which remain totally or almost unaltered on a larger scale of time. Our purpose was to evaluate how the easily, and free-of charge-accessible spatial databases may be used to derive landscape factors by predicting spatial patterns of roe deer roadkills on the Hungarian M3 highway. The result of the generalized linear model suggested that, despite the fact that six of the implemented variables were considered to be highly significant, none of them had a remarkable impact on the roadkills' spatial pattern, since their coefficient (B-value) were in each cases almost equal to zero. We concluded that existing landscape databases which had been used in this work are not suitable to support roadecology-decisions alone, but may have a supplementary role. This consequence put the weight to the other possible predicting factors (such as traffic-, and human related factors), and emphasizes the importance of the proper mitigation measures, and well maintained protective fencing, taking into special account that temporary dysfunctions of the protective fencing may lead to occasional – and so unpredictable – wildlife occurrences on highways.

**Keywords:** roe deer-vehicle collision, roadkill patterns, Network Kernel Density Estimation, highway, road ecology

### INTRODUCTION

Wildlife Vehicle Collisions (WVCs) cause annually more than 30 million forints damage to property in average on the Hungarian M3 highway, officially. This number in 2010 exceeded 54 million forints (according to police data on WVCs). These numbers are made worse by light, and serious personal injuries, and sometimes, deaths.

For practical point of view roe deer (*Capreolus capreolus*) is one of the most dangerous one for traveller's safety of the regularly occurring, conflicting wildlife species on the Hungarian highways. Its reason is, first of all, the relative big body mass. Additionally roe deer's long legs put most of its body to an almost equal level to most of the car's windscreens. Thus, in case of collision the probability for any kind of personal injury obviously increases.

Severity of roe deer collisions might be minimized by manipulating its influencing factors, so these factors are firstly to identify and recognize. In pursuit of this effort many scientific studies and synthesising work were done so far (PUTMAN, 1997; TROMBULAK AND FRISSELL, 2000; FORMAN, 2003; IUELL ET AL., 2003; IUELL, 2007; etc.). Influencing factors can be classified into different groups such as: temporal, traffic-related, landscape, weather, etc. (COLINO-RABANAL ET



AL., 2011; CARVALHO AND MIRA, 2010; GRILO ET AL., 2009; RAMP ET AL., 2005; SEILER, 2005).

Within this study we focus on the landscape features which remain totally or almost unaltered on a larger scale of time, too, such as settlements range, forested areas' range, hydrological features of the surface, etc.). Our purpose was to evaluate how the easily, and free-of charge-accessible spatial databases may be used to derive landscape factors by predicting spatial patterns of roe deer roadkills on the Hungarian M3 highway. Permanent wildlife-relevant features are expected to remarkably influence spatial patterns of roadkills.

## MATERIAL AND METHOD

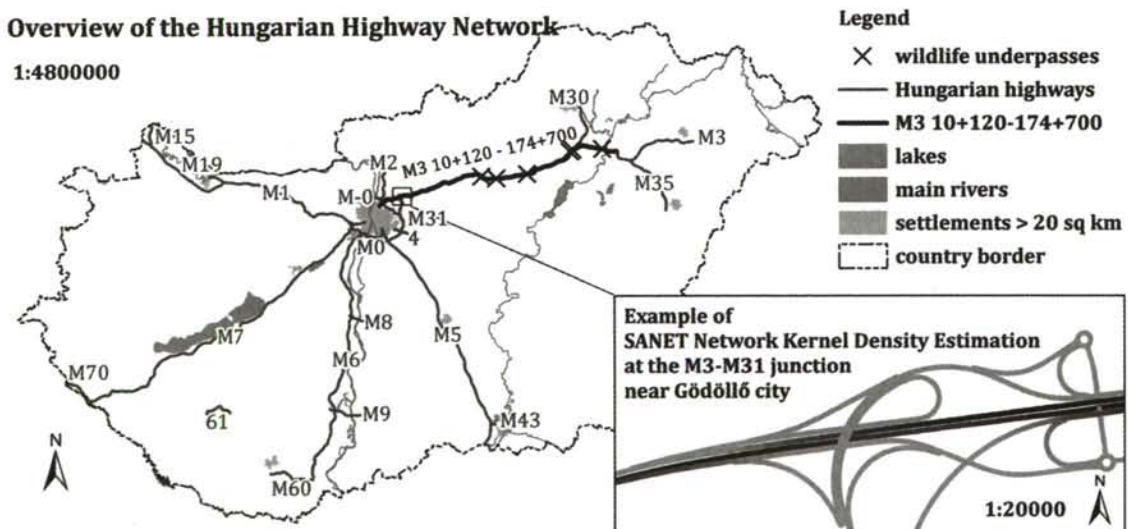
### Study area

Our study area was the section between the 10+120<sup>th</sup> and the 174+700<sup>th</sup> kilometres of the Hungarian M3 highway (Figure 1.). The M3 is one of the most important motorways of Hungary, a part of the so called "V<sup>th</sup>" or "Trieste – Venice – Ljubljana – Maribor – Budapest – Uzhhorod – Lviv - Kiev" Pan European transition corridor so it has a remarkable role in the East-West transportation. The first 70 kilometres of it was already built in 1983. The highway reached Füzesabony at the 114<sup>th</sup> kilometre in 1998 and Polgár at the 175<sup>th</sup> kilometre in the year 2002. The approximate daily traffic volume on this section exceeds 20000 vehicles per day. There are two traffic lanes, auxiliary lanes and one hard shoulder (emergency lane), as well; the speed limit is 130 km/hour.

The highway is located primarily along the frontier of the Great Hungarian Plain and the Northern Medium Mountains of Hungary. In the first part of the M3 hilly, whilst on the second part plain landscape dominates. Beside two forested area on the Eastern and the Western side of Gödöllő the adjacent of the road is mainly ruled by agricultural fields.

On the section was built before 1983 there are not any wildlife passages. Between the 80<sup>th</sup> and the 175<sup>th</sup> kilometres 6 different wildlife underpasses are located, but not any overpasses. According to the current Hungarian standards the whole M3 is surrounded by protective fencing (MAGYAR ÚTÜGYI TÁRSASÁG, 2007).

Spatial road data are deriving from the National Road Databank and were provided by the Coordination Centre for Transport Development of Hungary.



**Figure 1. Schematic overview and an example of the investigated highway section**  
**Data acquisition and analysis**



Roe deer roadkill data from 2003 till 2011 for the respective highway section were taken from the State Motorway Management Ltd.'s database. Landscape data are derived from the Corinne Land Cover 2006 (CLC 2006), and the National GIS Database, (OTAB 1, 1:100000).

The common GIS environment for the data handling and analyses was an ArcGIS 9.3 platform. Statistical calculations along a network such as river line or transportation infrastructure need slightly different approach than 2-dimensional, planar calculations. OKABE ET AL. (2006) improved a statistical application called SANET which provides more appropriate outputs in a network environment than standard planar statistics would do so. We used the SANET version 4 for the following network-statistical tasks. To investigate the degree to which clustering occurs within the roadkill dataset the network K-function (OKABE AND YAMADA, 2001) – which is an adaptation of Ripley's K-function – was calculated with 99 times of Monte Carlo simulations in order to ensure the statistical significance of the observed distribution. Network Kernel Density Estimation with discontinuous equal split method was used to measure the average roadkill density for each 50 metres. Bandwidth was set to 750 metres (so NKDE values apply to 1.5 km) road length according to the fact that roe deer individuals may have the chance for certain migration in short time along the highway within the fences. SANET's distance between base points and non-base points' method were used several times, as well. 3000 points were placed randomly along the network with a minimum distance of 100m between each other (mean:  $111 \pm 25.8$  SD). On each of these sample points all of the following list's variables' values were calculated in order to get a representative sample for the whole range of the road network. The list of the investigated variables is shown in *Table 1*.

**Table 1. List of the investigated variables with definitions and descriptive information**

variable name	definition	descriptive
<b>dependent variable</b>		
NKDE750	NKDE [roadkill density per 1.5 km]	$0.22 \pm 0.417$ ; min: 0.000; max: 4.714
<b>explanatory variables</b>		
D_VEGTOL	distance to nearest access road joint [m]	$2927 \pm 1955.3$ ; min: 1; max: 9208
D_ALULJ	distance to nearest under-crossing structure [m]	$1273 \pm 1119.3$ ; min: 0; max: 6250
D_FELULJ	distance to nearest over-crossing structure [m]	$695 \pm 515.5$ ; min: 0; max: 3036
D_VADATJ	distance to nearest wildlife underpass [m]	$20348 \pm 21638.9$ ; min: 9; max: 70875
D_ERDO	distance to nearest forest edge [m]	$619 \pm 521.7$ ; min: 0; max: 3022
D_TELEP	distance to nearest settlement's edge [m]	$1345 \pm 817$ ; min: 0; max: 4540
A_RI500	$\alpha$ -Richness*	min: 1; max: 6
SHAD500	Shannon's Diversity Index*	$0.46 \pm 0.415$ ; min: 0; max: 1.544
%ERDO_500	proportion of forested areas* [%]	$7.6 \pm 20.98$ ; min: 0; max: 100
%TERM_500	proportion of natural like areas* [%]	$2.2 \pm 6.52$ ; min: 0; max: 43.2
%NYIT_500	proportion of opened areas* [%]	$75.7 \pm 32.41$ ; min: 0; max: 100
VIZ_500	water presence/absence*	1: "yes" or 2: "no"
A_RI2500	$\alpha$ Richness**	min: 1; max: 12
SHAD2500	Shannon's Diversity Index**	$0.97 \pm 0.475$ ; min: 0; max: 1.952
%ERDO_2500	proportion of forested areas ** [%]	$6.8 \pm 14.64$ ; min: 0; max: 76.9
%TERM_2500	proportion of natural like areas** [%]	$3.4 \pm 3.31$ ; min: 0; max: 14.9
%NYIT_2500	proportion of opened areas** [%]	$72.2 \pm 23$ ; min: 6.7; max: 100
VIZ_2500	water presence/absence**	1: "yes" or 2: "no"

\*within 500 m radius, \*\*within 2500m radius

Distances were measured by Euclidean distance method.  $\alpha$ -Richness and Shannon's Diversity Indices were calculated by using Diversity Calculator by (BUJA, 2009) based on each CLC categories.



The class “forested areas” (%ERDO\_500; %ERDO\_2500) contains CLC categories of 3.1.1: broad-leaved forest; 3.1.2: coniferous forest; 3.1.3: mixed forest. Into the class of “natural-like areas” (%TERM\_500; %TERM\_2500) belong CLC 2.4.3: land principally occupied by agriculture, with significant areas of natural vegetation; 3.2.1: natural grasslands; 3.2.4: transitional woodland-shrub; and 4.1.1: inland marshes. “Opened areas” contain CLC 2.3.1: pastures, and 2.1.1: non-irrigated arable land. Water may indicate watercourses and water bodies, equally.

The two distance threshold values (500m and 2500m) reveal two different background hypotheses: taking into account the landscape parameters within 500m would mean that we expect individuals that were roadkilled at their normal daily activity (500m radius derives from the area of an average spring-autumn home range size), since the landscape parameters within 2500m must be considered in case of supposition of long-distance moving, exploring, dispersing individuals (CSÁNYI ET AL., 2009). Spearman’s nonparametric correlation was run to identify the correlations between any of the explanatory variables. Effects on explanatory variables on the response were evaluated by a generalized linear model with 1.5 parameter tweede distribution with “log” link function. Data management and the statistical analyses were carried out by the software PASW 18.0 (SPSS Inc.), the R statistical package, and Microsoft Office 2007.

RESULTS

Between 2003 and 2011 there were 115 roe deer carcasses found on the M3 highway from its beginning till the 175<sup>th</sup> kilometre. According to the Network K-function curves the distribution of these roadkills’ locations along the road network show significantly clustered pattern below a distance of approximately 80km (Figure 2.).

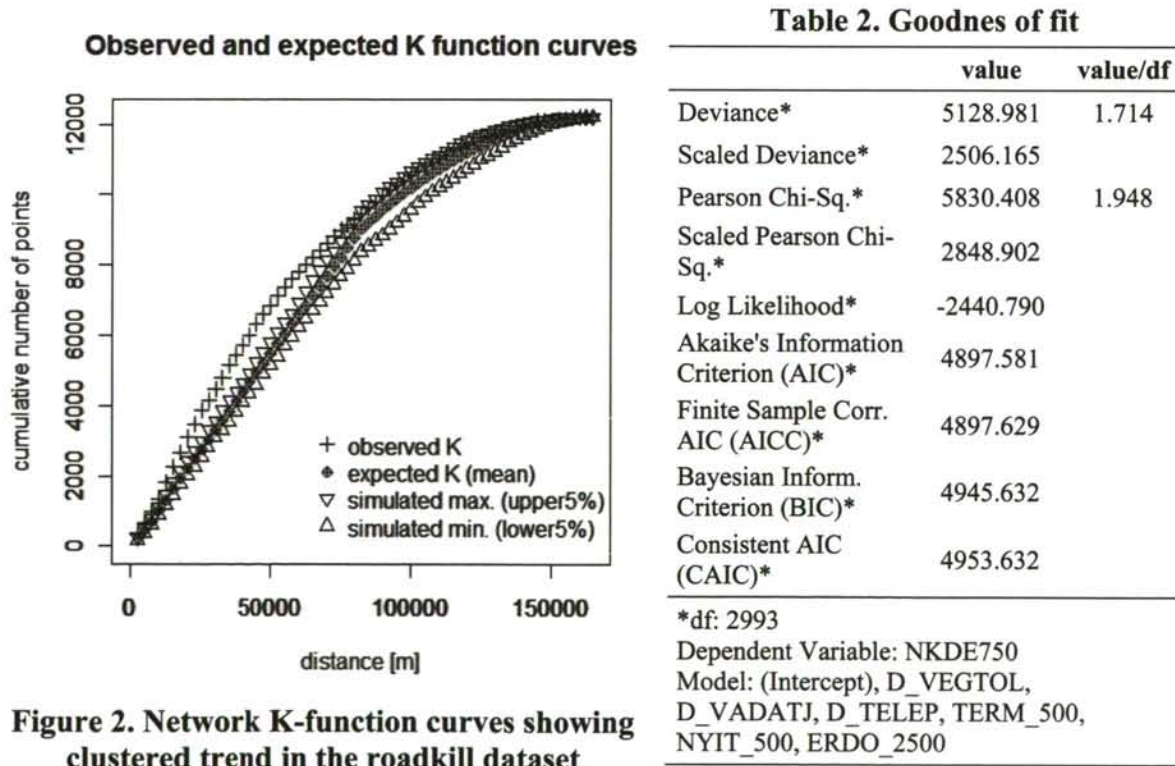


Figure 2. Network K-function curves showing clustered trend in the roadkill dataset

The Spearman's Rho Nonparametric Correlation pointed out a few highly significant ( $p < 0.01$ ), strong (correlation coefficient  $> 0.71$ ) correlation. In these cases, in order to reduce the autocorrelation we excluded one of the variables, respectively: A\_RI500, A\_RI2500, SHAD500, SHAD2500, %ERDO\_500, %NYIT\_2500. Furthermore the variable VIZ\_500 found to be extremely unbalanced, consequently was not used longer in the model. We run the model with an iteration method, and step by step eliminated the following non-significant variables: %TERM\_2500, D\_FELULJ, D\_ALULJ, D\_ERDO, VIZ\_2500. The final model is summarized in *Table 2*. The fitted model (*Table 3*.) was significantly differing from the intercept-only model by the Omnibus test (Likelihood Ratio Chi-Square: 139.435; df: 6;  $p < 0.001$ ).

**Table 3. Parameter estimates**

parameter	coefficient (B)	std. error	Wald	df	p
(Intercept)	-1.515	.1865	65.930	1	<0.001
D_VEGTOL	.000	.0000	8.275	1	0.004
D_VADATJ	.000	.0000	25.616	1	<0.001
D_TELEP	.000	.0001	61.583	1	<0.001
TERM_500	.022	.0056	15.949	1	<0.001
NYIT_500	-.008	.0019	19.393	1	<0.001
ERDO_2500	-.022	.0042	28.734	1	<0.001
(scale)	2,047 <sup>a</sup>	.0492			

dependent Variable: NKDE750  
 modell: (Konstanter Term), D\_VEGTOL, D\_VADATJ, D\_TELEP, TERM\_500, NYIT\_500, ERDO\_2500

a. Maximum-Likelihood-Estimator

## CONCLUSIONS

The Network K-functions' curves showed the clustered trend along the road network in the roadkill dataset. The chosen landscape factors, however, found to be highly significant; do not have much predictive power to the spatial patterns of the roadkill hotspots; since their coefficient (B-value) are almost equal to zero. This consequence put the weight to the other factors (such as traffic-, and human related factors), and emphasizes the importance of the protective fencing and the mitigation measures.

In accordance to our study's aim we must conclude that the existing landscape databases which had been used in this work are not suitable to support road ecology-decisions alone, but may have a supplementary role. However, with additional data, existing information might be refined, and so with a synergist contribution to the result.

Results of this work are in line with our earlier suggestions in terms of the highway-management: protective fences, service gates, and crossing structures have always to be well maintained (MARKOLT ET AL., 2009). Temporary dysfunctions (open left service gate, cut fencing) of the fencing may lead to occasional roe deer and other animal's occurrences on the protected side of the highway, and may threat the traffic safety. Accidents and roadkills which are resulted by this reason are not predictable by landscape factors.

Finally an interesting result is that the assumed impact of the 6 existing underpass (as permanent wildlife-relevant features) was not observable. One of its explanations could be that these passes are not functioning well, but this hypothesis needs further investigations.



## ACKNOWLEDGEMENTS

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## THE ABSOLUTE SPECIALISATION OF HUNGARIAN INDUSTRY WITH REGARD TO REGIONAL EXPORT

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### **ABSTRACT – The absolute specialisation of Hungarian industry with regard to regional export**

In the European Union member states, including Hungary, the spatial and structural texture of industry transformed considerably during the past few years. We can encounter both spatial and sectoral winners and losers of this transformation. It occurred that the number of the employed in certain industrial sectors decreased, domestic and export sales occasionally dropped, and in case of other industrial sectors the opposite emerged. This raises the necessity of tests, with the help of which it can be determined whether there was any concentration increase or decrease in the spatial organization of industries that in recent years, and if the marked change in industrial specialization of the counties was characteristic.

To ensure that industrial changes of recent years (plot, structure) can be monitored, it is important to examine the spatial concentration of industries in Hungary and the industry specialization over time in each area. This study presents the development of the absolute spatial specialization of the Hungarian industry in the light of export sales.

**Keywords:** industry, specialization, Herfindahl index, export sales

## INTRODUCTION

The spatial and structural texture of Hungarian industry is due to a long development dating back several decades. The spatial location of certain industries was influenced by several social-economical factors (natural-social-economical-political) in the past few decades resulting in a peculiar spatial industrial structure.

The examinations of the spatial concentration and of the sectoral specialisation of the regions have considerable history both in Hungarian and international professional literature (GREENWAY – HINE, 1991; AMITI, 1997; BRÜLHART, 1998). A great number of studies appeared concerning the territorial situation of Hungarian industry in the decades preceding the regime change, which focused on the micro and mezzo-levels examinations instead of macro levels (KÓRÓDI – MÁRTON, 1968; BARTKE – BORA – ILLÉS, 1971; ABONYINÉ ET AL. 1976; TATAI, 1984). The examination of the various industrial sectors received different attention. In most cases the research focused on food industry (Abonyiné, 1983; Gurzó, 1986), as well as on the issues of textile industry, machine industry and building material industry (BENCZE, 1962; ANTAL – FÜLÖP, 1973).

The examinations highlighted that the endowments and the production traditions of the various territories and regions were different from each other and consequently the spatial work distribution is rather different appearing particularly in the production profile of the certain territories. Therefore “specialisation is the most important process in becoming a region, which is resulted by the naturally not equal formulation and development of several production sectors (KRAJKÓ ET AL. 1969).



In the years following the regime change geographical location and the changes of industry once again became the focus of the research, since Hungarian industry has undergone significant changes. Our economic relations from one day to the next one become west oriented instead of having eastern orientation. This provided an opportunity that our country can be part of the global market and through that the part of the global economy. Consequently the market conditions, the production and distribution facilities changed, resulting in a completely new situation in the sectors of national economy, which meant new challenges in specialization, especially in the field of spatial specialization. In some sectors of the economy and industry sub-sectors the volume of production declined, the employment structure transformed, the production structure became irrational, the composition more heterogeneous and sometimes sales difficulties occurred.

As a result of these changes the examinations in the territorial situation and the structure of industry started again (ANTAL, 1993; BARTA, 2002; RÉDEI – JAKOBI – JENEY, 2002; HORVÁTH, 2002; BODNÁR – HORVÁTH, 2005; KISS, 2010; ABONYINÉ – KOMAREK, 2011). Both in Western Europe and in Hungary the quantitative examinations concerning the spatial concentration of industries and the specialization of the territories showed that spatial concentration took place in many industrial sectors in the 1980s and 1990s. The spatial concentration was associated with the specialization of the industrial activity of certain territories (regions, counties).

## **MATERIAL AND METHOD**

There are several indices to measure the industrial specialisation of the counties (eg. Specialisation index, spatial specialisation index, Herfindahl-index, Theil-index). Out of the various indices I selected the Herfindahl index (absolute specialisation) for my research, as it is suitable for determining the spatial distribution of the industrial specialisation of the counties by their export trade.

Specialisation measuring:

Herfindahl index (absolute specialisation):

$$H_j^S = \sum_i (S_{ij}^S)^2$$

where:

i = industry

j = county

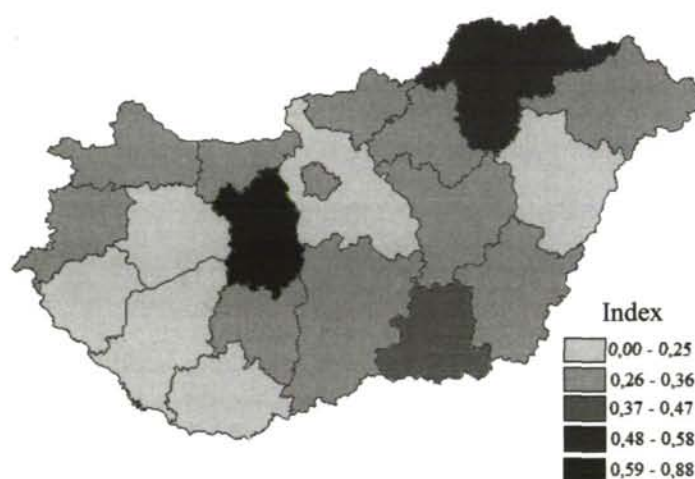
$S_{ij}^S$  = j county i industry share from j county total export trade

The Herfindahl index value can be between 0 and 1. The higher the value of the absolute specialisation is, the higher the level of the absolute specialisation will be.

The data source for the research was the available data of industrial organizations situated in the counties provided by the Central Statistical Office (CSO = Central Statistical Office). The examined dates were the years of 1992, 1998, 2000 and 2008 due to the frequent changes of the TEÁOR (Tevékenységek Egységes Ágazati Osztályozási Rendszere = Single Classification of Industrial Activities) numbers. These data allow comparisons and conclusions concerning the export sales of industrial specialization in the counties.

## RESULTS AND CONCLUSIONS

According to the calculations the following statements can be made. In 1992 a high index value was observed in case of Fejér, Borsod-Abaúj-Zemplén and Csongrád Counties. The high index value of Fejér County was due to the manufacturing of metallic raw material and metal processing products, while that of Borsod-Abaúj-Zemplén County was due to the chemical industry and in Csongrád County it was due to the food, drink, and tobacco production. Baranya, Somogy, Zala and Hajdú-Bihar Counties, among others, belonged to the ones with low index values. The reason for this was the lack of an industry in the given county, which could represent a significant proportion in the total export trade of the county, which means a more complex industrial export structure is characteristic, and also the decrease of the previously dominant food industry caused this lower index value (Figure 1.).

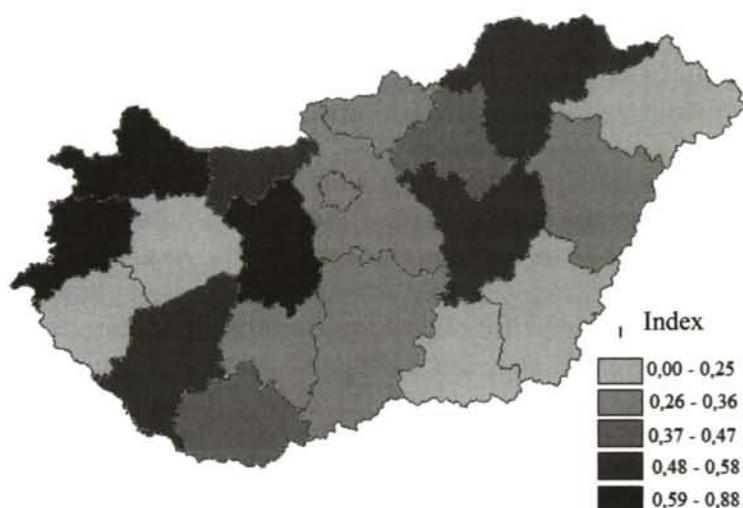


**Figure 1. The spatial distribution of the absolute specialisation of the Hungarian industry by export trade (1992)**

Edited: by the author from CSO data

In 1998 Borsod-Abaúj-Zemplén County kept its „leading position” in the export trade concerning the chemical industry. In the same year a high index value was observed in case of Fejér County as well, however the leading industrial sector changed here during the seven years: instead of the manufacturing of metallic raw material and metal processing products, which used to stand on the first place, the machine industry became dominant. The upgrading role of the machine industry is shown by the fact that the given industrial sector became dominant, regarding export trade, not only in Fejér County but also in numerous other Transdanubian counties (Győr-Moson-Sopron, Vas, Somogy, Komárom-Esztergom). In the background of the largest increase in absolute specialisation was mainly the machine industry (Heves, Jász-Nagykun-Szolnok, Komárom-Esztergom, Baranya, Vas, Somogy, Győr-Moson-Sopron) (Figure 2.). In addition to the above-mentioned counties, the index increased in Hajdú-Bihar County as well, which was due to the growing importance of food industry. At the same time, from 1992 to 1998 specialization decreased in three counties (Csongrád, Békés, Szabolcs-Szatmár-Bereg). This was caused by a decreasing dominance of the food industry.

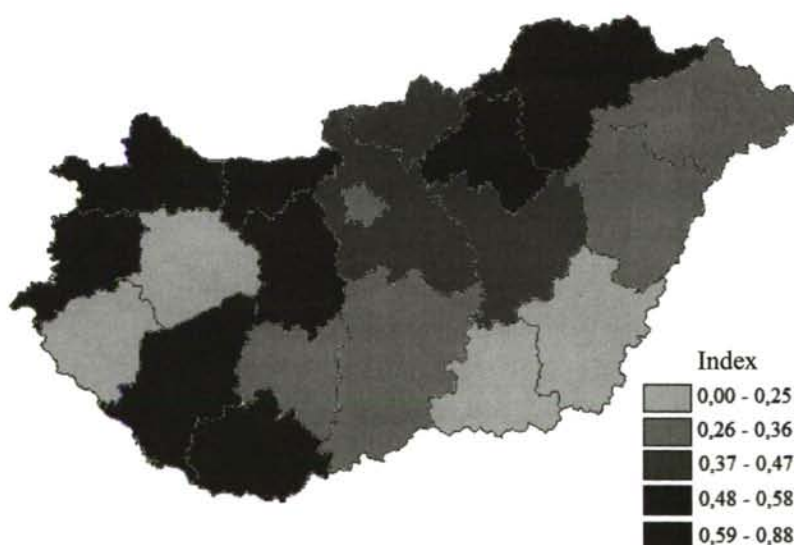




**Figure 2. The spatial distribution of the absolute specialisation of the Hungarian industry by export trade (1998)**

Edited: by the author from CSO data

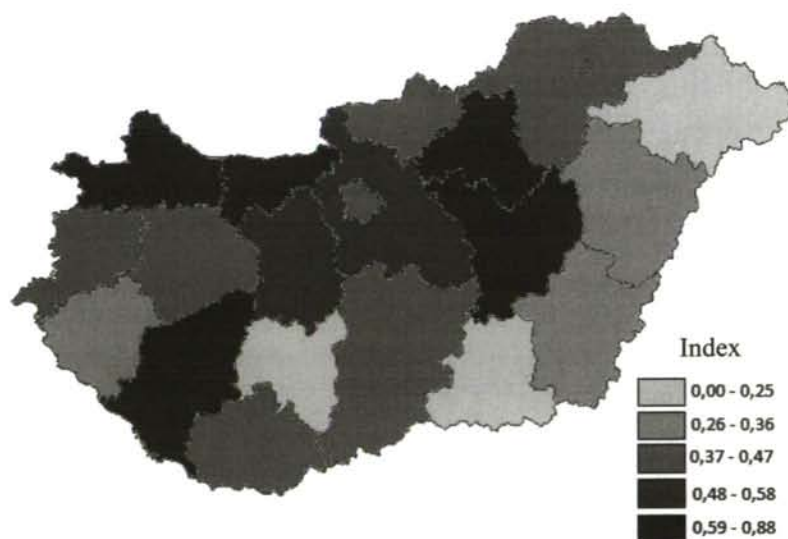
In 2000 there was a high absolute specialisation in Fejér, Győr-Moson-Sopron, Komárom-Esztergom, Vas and Somogy counties. The high index values were due to the machine industry and the manufacturing of metallic raw material and metal processing products in Fejér County, while in Győr-Moson-Sopron, Komárom-Esztergom, Somogy and Vas counties they were due to the machine industry. It was found, that the export of machine industry in the above mentioned counties was over 80% (except for Vas county – 78.2%). This means the given counties are specialised in machine industry regarding export trade. Borsod-Abaúj-Zemplén County also must be mentioned, despite the fact that it does not belong to the group of the 5 counties with the highest indices. This county deserves special attention being the only one in 2000 specialised in chemical instead of machine industry (74% of the export of the county was given by the chemical industry).



**Figure 3. The spatial distribution of the absolute specialisation of the Hungarian industry by export trade (2000)**

Edited: by the author from CSO data

The last counties in the rank of export trade are Zala, Veszprém, Csongrád and Békés. In their case there is no dominant industrial sector with a significant role in the export trade of the given county (Figure 3.). By 2008, when compared to 2000, there was a decrease in Fejér County (out of the previously mentioned), and in Győr-Moson-Sopron County a slight (0.01) decrease was observed (Figure 4.). Komárom-Esztergom County increased its advantage, which resulted in a 0.88 increase in the value of the index. This improvement was again due to the machine industry. The cause of the fall-back of Fejér County is the fact that the importance of the machine industry decreased, while the role of the manufacturing of metallic raw material and metal processing as well as the non-metallic mineral product manufacturing increased in export trade. In the given year Komárom-Esztergom County as well as Heves and Jász-Nagykun-Szolnok Counties proceeded in the rank. Both Heves and Jász-Nagykun-Szolnok Counties got a better position because of the increasing importance of the machine industry. In case of Heves County the proportion of machine industry export in 2000 was 70.9% of the total and it increased to 91.0% by 2008. In Jász-Nagykun-Szolnok County the proportion of machine industry export in 2000 was 64.8% of the total county export, while 82.5% in 2008. It reflects a rather strong specialisation in the field of machine industry. (Special attention should be devoted also to Borsod-Abaúj-Zemplén County in this very same year, where the export trade of chemical industry increased, at the same time the proportion within the industry of the county decreased, which can be explained by the dynamic expansion of the proportion of the machine industry). The lowest index values of the 2008 year belong to Szabolcs-Szatmár-Bereg, Tolna and Csongrád Counties. The reason for this can be the lack of a dominant industrial sector, i.e. a more versatile industrial export structure is characteristic.



**Figure 4. The spatial distribution of the absolute specialisation of the Hungarian industry by export trade (2008)**

Edited: by the author from CSO data

To sum up, in the examined period the increase of the importance of the role of machine industry might be the cause of the increase of the Herfindahl index, among others between Fejér, Győr-Moson-Sopron, Vas, Komárom-Esztergom, Heves and Jász-Nagykun-Szolnok counties. The decrease of the index was resulted by the multi-polar export trade as well as



the ceasing dominance of food, drink and tobacco production among others between Csongrád, Békés and Szabolcs-Szatmár-Bereg counties.

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## BIO-ECONOMIC IMPACT OF ENERGY AND PROTEIN LEVEL IN FEED FOR LAYING HENS RAISED IN ORGANIC SYSTEM

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### ABSTRACT

The economic results of organic egg production are largely dependent on the cost of the feed and productive performance. The paper aims to establish the productive performance of laying hens raised under specific conditions specific for the ecological system, and also performing a feeding costs estimation based on mathematical models starting from experimental data required. The hens in the experiment were fed a mixture of concentrated (MC) with 2728 kcal metabolizable energy (ME), 15.85% crude protein (CP), 0.67% lysine, 0.52% methionine + cystine, containing in its structure only fodder types organically certified. Throughout the entire experimental period, chickens have recorded a total MC consumption of 13.240 kg with an average daily consumption between 0.110 and 0.130 kg, when they produced a daily average of 31.55 g mass-egg. The amount of mass-egg produced ( $y$ ) under this experiment can be predicted based on metabolizable energy intake ( $x_1$ ) and crude protein ( $x_2$ ) using the following mathematical model  $y = a + b \cdot x_1 + c \cdot x_1^2 + d \cdot x_1^3 + e \cdot x_1^4 + f \cdot x_1^5 + g \cdot x_2$  ( $R^2 = 0.99\%$ ). Between the values obtained after the experiment and the predicted values obtained using the mathematical relationship, the differences are very small 2.17% at the end of experimental period. The costs incurred by feeding the laying hens ( $y$ ) organically raised can be predicted using the following mathematical model  $y = \exp(a \cdot x_1 + b \cdot x_2 + c \cdot x_3 + d)$  ( $R^2 = 0.96$ ), with a rate of error of less than 2.2%, depending on the amount of mass-egg produced ( $x_1$ ), metabolizable energy intake ( $x_1$ ) and crude protein intake ( $x_2$ ).

**Key words:** productive performance, mathematical model, costs of feeding, organic poultry

### INTRODUCTION

Organic poultry could be one of the agricultural opportunities of development of agriculture in Romania since the market demand for organic products is increasing (ORBOI, 2009). Organic farming can make a significant and lasting economic development and plays an important role in improving the environment and water quality, bio-diversification and nature protection (BLAIR, 2008, DE BOER, 2002, FERRANTE, 2009, MERTENS, 2009).

The economic performance of organic egg production is largely dependent on the price of feeding and on the productive performance (SUNMER, 2010). Since feed costs are the most important economic factor, it should be closely monitored to ensure a maximum efficiency (WEERSINK, 2002). Thus, from this point of view, feeding costs represent over 90% of total variable costs.

Organic table birds and layers must be produced in accordance with the standard practices set out by the European Council Regulations and monitored by the certifying bodies in each country (EC, 2007, 2008, 2009).

The work proposes to set the productive performances of laying hens fed under required conditions specific to ecological system, and also predicts the feeding costs according to some mathematical methods starting from the experimental data obtained.



## MATERIAL AND METHODS

The experiment was performed to obtain organic eggs on a total of 90 hybrid ISA BROWN hens aged between 20-36 weeks, in accordance with the Community legislation ensuring them food and maintenance conditions specific to organic system.

Hens in the experiment were fed ad libitum with a mixture of concentrated (MC) with 2728 kcal ME, 15.85% CP, 0.67% lysine, 0.52% methionine + cystine, its structure containing only forage varieties organically certified.

The experiment lasted for 16 weeks (October-January) during the ascendant phase and during a period of plateau phase of the laying curve.

Bio performance of hens was assessed using the following indicators:

- MC intake, determined weekly and expressed by MC consumption (kg/wk/hen) and average daily consumption (ADC),
- The development of the egg-mass-, based on the number of eggs produced weekly and according to their weight,
- Feed conversion ratio expressed in kg MC/kg egg-mass,
- Energy intake (kcal ME/kg egg-mass) and protein (g/kg egg-mass), determined on the use of MC.

Recorded initial data were processed statistically using the computer program SPSS 19. Based on energy and protein intake using informational software Data Fit 9, have been established equations of prediction of egg-mass quantity specific of that class of birds and also prediction using mathematical models for feeding costs registered.

## RESULTS AND DISCUSSION

After performing the experiment regarding the bioproductive parameters evaluation in laying hens reared in ecological system and under the specified condition, there were obtained the following results:

- *MC intake* data are presented in *Table 1*.

**Table1. Evolution of feed consumption in laying hens bred in organic system.**

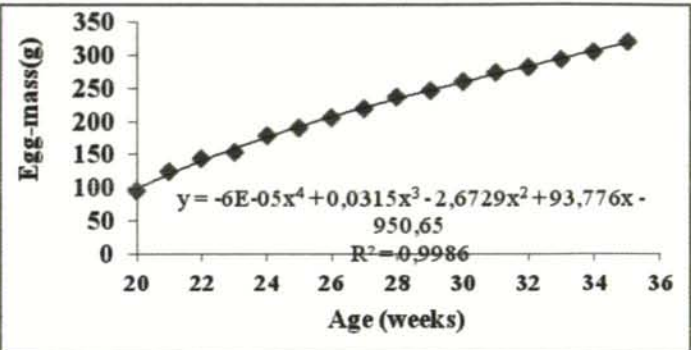
<i>Age (weeks)</i>	<i>MC intake (kg)</i>		<i>Average daily consumption (kg)</i>
	<i>/period</i>	<i>cumulated</i>	<i>/period</i>
22	1.535	1.540	0.110
24	1.535	3.070	0.110
26	1.580	4.650	0.110
28	1.624	6.270	0.120
30	1.673	7.950	0.120
32	1.780	9.730	0.130
34	1.750	11.470	0.120
36	1.765	13.240	0.130

As shown in *Table 1*, that has registered a total consumption of 13.240 kg AC throughout the experimental period, the average daily consumption (ADC) between 0.110 kg in the first period and 0.130 kg in the last experimental period.

– *evolution of mass-egg* Initial data on mass-egg are presented in Table 2. The data in Table 2 on egg-mass obtained from hens of the experiment show that it increases from 15.72 g/hen/day at the age of 20 weeks up to 44.70 g/hen/day at the age of 36 weeks.

**Table2. The evolution of egg-mass in laying hens in this experiment**

Age (weeks )	Average /hen/day	Egg-mass g/hen/period
22	15.72	220.06
24	21.21	296.93
26	26.43	370.05
28	30.44	426.21
30	34.66	485.28
32	38.12	533.73
34	41.17	576.37
36	44.70	625.85
		<b>3534.48</b>



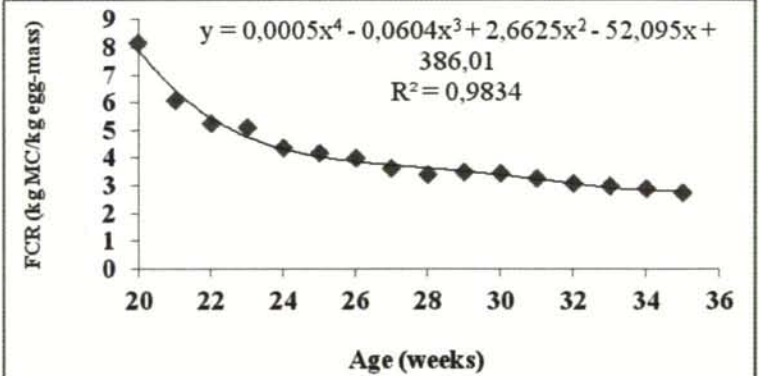
This increase of egg-mass quantity is bigger in the first weeks of the experiment, weeks coinciding with the ascendant phase of the laying curve, followed by a moderate increase when hens reach the plateau phase of the laying curve.

Throughout the experimental period, the amount of egg-mass produced was of 3534.48 g/hen, which is an average of 31.55 g egg-mass/hen/day.

– *the feed conversion ratio (FCR)* with fortnightly ratings is presented in Table 3 and graphically traced, modeled using the polynomial regression of fourth degree in the figure inserted in Table 3.

**Table 3. The evolution of the feed conversion ratio (kg MC/kg egg-mass)**

Age (weeks)	Feed conversion ratio/period
22	6.06
24	5.09
26	4.17
28	3.63
30	3.50
32	3.24
34	2.97
36	2.76
<b>Total</b>	<b>4.12</b>



Throughout the period under review there was a feed conversion ratio of 4.12 kg MC/kg of egg-mass. At the beginning of experimental period that coincides with the beginning of laying process, the conversion ratio is higher due to the reduced production of mass-egg. The aim is downward as hens lay more eggs, so in the last week the experimental FCR is only of 2.76 kg MC/ kg egg-mass.

The coefficient of determination ( $R^2$ ) of the polynomial regression equation of fourth degree, which helped us to graphically represent the conversion index with birds' age as a ratio, was higher (98.34%).



– *average daily energy and protein intake*

On the basis of the average daily consumption of MC and its content in nutritional criteria, there was determined the weekly intake of metabolizable energy and crude protein.

Average daily intake of metabolizable energy ranged between 205.60 kcal/kg egg-mass in the first experimental week and 241.25 kcal/kg egg-mass in the last experimental week.

Average crude protein intake was between 11.95 g/kg egg-mass and 14.02 g/kg egg-mass, which is variable depending on the experimental week and definitely of the amount of egg-mass.

We followed the development of egg-mass equations based on energy and crude protein intake. With the help of the informational soft Data fit 9.0 there were obtained several mathematical models, of which we chose to present the equation with the highest multiple coefficient of determination ( $R^2$ ) and the lowest percentage of error for the entire experimental period.

The type of equation is:  $y = a + b \cdot x_1 + c \cdot x_1^2 + d \cdot x_1^3 + e \cdot x_1^4 + f \cdot x_1^5 + g \cdot x_2$ , where:

$y$ - the amount of mass-egg,

$x_1$ - the metabolizable energy intake (kcal ME)

$x_2$ - crude protein intake (g).

The resulted mathematical model coefficients and its statistical indices are presented in Table 4.

**Table 4. Coefficients and statistical indices of mathematical equation of prediction for mass-egg according to metabolizable energy and crude protein intake**

<i>Coefficient</i>	<i>Value</i>	<i>St. error</i>	<i>t-ratio</i>	<i>Prob(t)</i>
a	1.19E+08	17137361	6.920391	0.00007
b	-2648323	381952.6	-6.93364	0.00007
c	23631.16	3403.48	6.943232	0.00007
d	-105.337	15.15239	-6.95187	0.00007
e	0.234557	3.37E-02	6.958677	0.00007
f	-2.09E-04	3.00E-05	-6.96367	0.00007
g	1892.864	3039.428	0.62277	0.54889

$$Y = 1,19E+08 + -2648323 \cdot EM + 23631,16 \cdot EM^2 + -105,337 \cdot EM^3 + 0,234557 \cdot EM^4 + -2,09E-04 \cdot EM^5 + 1892,864 \cdot PB$$

*Variance Analysis*

<i>Source</i>	<i>DF</i>	<i>Sum of Squares</i>	<i>Mean Square</i>	<i>F Ratio</i>	<i>Prob(F)</i>
Regression	6	64575.56	10762.59	18.08398	0.00015***
Error	9	5356.306	595.1451		
Total	15	69931.87			

After applying this mathematical equation, we can predict egg-mass in laying hens kept in organic system under similar conditions with those carried out in this experiment (Table 4). The analysis of these data show that the calculated values (egg-mass) are similar to those obtained experimentally during the 16 weeks. Model error is small, and at the end of the tested period the recorded error rate was of about 2.18%.

Another indicator followed in this study was a bio-economic analysis based on athemathical models of prediction of feeding expenses within this system of laying hens breeding.

Because feeding costs constitute a significant percentage of the total recorded production of eggs, we followed the development of models of prediction for expenditure necessary for hens feeding under the ecological breeding system, according to intake of metabolizable energy, crude protein and also according to the quantity of egg-mass.

With the help of informational soft Data fit 9.0, we have obtained three mathematical models presented in *Table 5*.

**Table 5. Mathematical prediction models for feeding costs per bird**

Rank	Model	StdError	Residual Sum	Residual Avg.	RSS	R <sup>2</sup>
1	$\exp(a \cdot x_1 + b \cdot x_2 + c \cdot x_3 + d)$	0.01811	7.00E-05	4.37E-06	0.00393	0.96684
2	$a \cdot x_1 + b \cdot x_2 + c \cdot x_3 + d$	0.01868	-8.88E-16	-5.55E-17	0.00419	0.96472
3	$a \cdot x_1 + b \cdot x_2 + c \cdot x_3$	0.02429	0.00718	0.00045	0.00767	0.93536

$x_1$ - egg-mass quantity,  $x_2$ - energy intake,  $x_3$ - protein intake,  $y$ - ingested feed cost.

The data analysis presented in *Table 5* show that all three mathematical models from processing experimental data recorded a coefficient of multiple determination ( $R^2$ ) higher than 0.93.

We chose to predict the feeding costs recorded using the first mathematical model resulted, with the highest coefficient of determination and the lowest percentage of error.

Coefficients and statistical index of the resulting mathematical equations ( $R^2=0.966$

$y = \exp(a \cdot x_1 + b \cdot x_2 + c \cdot x_3 + d)$  are presented in *Table 6*.

**Table 6. The coefficients and statistical indices of mathematical equation for prediction of feeding costs**

Coefficient	Value	St. error	t-ratio	Prob(t)
a	0.00035	0.00011	3.11172	0.00899
b	0.00253	0.00094	2.70288	0.01921
c	0.00187	0.01253	0.14940	0.88372
d	-0.27652	0.10046	-2.75251	0.01752
$y = \exp(0.00035 \cdot x_1 + 0.00253 \cdot x_2 + 0.00187 \cdot x_3 + -0.27652)$				

#### Variance Analysis

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob(F)
Regression	3	0.1147	0.0382	116.6234	0.0000
Error	12	0.0039	0.0003		
Total	15	0.1186			

$y$ - ingested feed cost,  $x_1$ -produced mass-egg quantity,  $x_2$  energy intake,  $x_3$  protein intake.

The data analysis presented in *Table 6* show that between the experimental and predicted data obtained under this equation, the differences are very small throughout the experimental period. Throughout the production of eggs produced in the 16 experimental weeks, feeding costs were of 23.835 lei (5.48 €), which is 6.744 lei/kg egg-mass (1.55 €), value resulting from the application of the mathematical equation presented in *Table 6*.

Following the application of these mathematical equations, we can determine the cost of laying hens feeding kept in organic system under conditions similar to this experiment, after setting the metabolizable energy intake, crude protein and egg-mass quantity.

The analysis of these data show that the calculated values (predicted price) are close to those obtained experimentally during the 16 weeks. Model error is smaller, resulting a percentage of error less than 2.2%.

## CONCLUSIONS

Hybrid ISA BROWN hens aged between 20 and 36 weeks bred in organic system and fed under required conditions, have registered a total consumption of MC 13.240 kg



with a daily average consumption between 0.110 and 0.130 kg, a period that produced a daily average of 31.55 g mass-egg.

The amount of egg-mass produced ( $y$ ) under conditions similar to this experiment can be predicted based on metabolizable energy intake ( $x_1$ ) and crude protein ( $x_2$ ) using the following mathematical model  $y = a + b \cdot x_1 + c \cdot x_1^2 + d \cdot x_1^3 + e \cdot x_1^4 + f \cdot x_1^5 + g \cdot x_2$  ( $R^2 = 0.99\%$ ).

Between the values obtained experimentally and the predicted values obtained using the mathematical relationship, the differences are very small: 2.17% at the end of experimental period.

Costs incurred by feeding organic laying hens kept in organic system can be predicted using the following mathematical model  $y = \exp(a \cdot x_1 + b \cdot x_2 + c \cdot x_3 + d)$  ( $R^2 = 0.96$ ), with a error percentage lower than 2.2%.

### ACKNOWLEDGMENTS

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- \*\*\* REGULAMENT (CE) NR. 834/2007 AL CONSILIULUI din 28 Iunie 2007 privind producția ecologică și etichetarea produselor ecologice, precum și de abrogare a Regulamentului (CEE) nr. 2092/91.
- \*\*\* REGULAMENT (CE) NR. 889/2008 AL COMISIEI din 5 septembrie 2008 de stabilire a normelor de aplicare a Regulamentului (CE) nr. 834/2007 al Consiliului privind producția ecologică și etichetarea produselor ecologice în ceea ce privește producția ecologică, etichetarea și controlul.
- \*\*\* REGULAMENTUR (CE) NR. 953/2009 AL COMISIEI din 13 octombrie 2009 privind substanțele care pot fi adăugate cu anumite scopuri nutriționale în produsele alimentare destinate unei alimentații speciale.



## EXTRACELLULAR LIPASE PRODUCTION OF ZYGOMYCETES FUNGI ISOLATED FROM SOIL

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### ABSTRACT

Filamentous fungi are well known by their ability to secrete biotechnologically important enzymes into the environment. Lipase enzymes catalyze the hydrolysis of triacylglycerols to give free fatty acids, diacylglycerols, monoacylglycerols and glycerol. There is a growing interest in microbial lipase production due to its great potential for various industrial applications. Zygomycetes are good producers of lipases, however, representatives of the order Mortierellales are poorly characterized from this aspect. Our knowledge in reference to the activity and production of the enzymes by soil isolated zygomycetous fungi is also limited. The main objective of this work was the screening of 35 soil isolated strains belonging to the genera *Mortierella*, *Dissophora* and *Umbelopsis* with potential to produce lipases. For detection of extracellular lipase production, culturing media containing tributyrin was used and the level of the lipase production was evaluated by measuring the diameter of the halo around the colonies. The halo was formed in consequence of the lipase activity and measured daily during the incubation period. The lipase production of the tested strains showed high variability and several isolates showing high enzyme activity were detected in each genus. Among the tested isolates, the *Dissophora ornata*, *Mortierella longicollis* and *Umbelopsis angularis* strains proved to be outstanding in their enzyme producing ability. The *M. longicollis* were selected to investigate the effects of various inductor oils on the enzyme production using submerged culture fermentation systems.

**Keywords:** Zygomycetes, lipase, tributyrin, microorganism screening, submerged culture fermentation

### INTRODUCTION

Lipases (glycerol ester hydrolases; EC 3.1.1.3) have multiple applications in a wide range of biotechnological processes. These enzymes catalyze the hydrolysis of triacylglycerols, which are the major constituents of fats and oils, to produce free fatty acids, glycerol and partial acylglycerols (SHARMA et al, 2001). This reaction is reversible, so that these enzymes also catalyse the formation of acylglycerols from glycerol and free fatty acids. There is a growing interest in microbial lipase production due to its great potential for industrial applications such as food additives (PETRUCCIOLI and FEDERICI, 1992), industrial reagents (JAEGER and REETZ, 1998) and stain removers, as well as for medical applications (KAZLAUSKAS and BORNSCHEUER, 1998). Lipases can also be used to accelerate the degradation of fatty waste and polyurethane (MASSE et al, 2001; TAKAMOTO et al, 2001). Filamentous fungi are well known by their ability to secrete biotechnologically important enzymes into the environment, e.g. mainly proteases and lipases. Filamentous fungi able to produce lipase enzymes can be found in some agro-industrial wastes, deteriorated foods as



well as different soil samples (COLEN et al, 2006; GRIEBELER et al, 2011). Zygomycetes are good producers of lipases and some *Mucor*, *Rhizomucor* and *Rhizopus* lipases have been isolated and utilized in the industry (SHARMA et al, 2001; NOEL and COMBES, 2003). However, representatives of the order Mortierellales are poorly characterized from this aspect, and our knowledge in reference to the activity and production of the enzymes by other soil-borne zygomycetous fungi is also limited. Therefore, 35 *Mortierella*, *Dissophora* and *Umbelopsis* strains isolated from different soil samples have been screened for their secreted lipase activity in order to find new producer isolates potentially applicable in further basic studies and biotechnological applications.

## MATERIAL AND METHOD

### Culture conditions

For the detection of lipase activity in plates, 20  $\mu$ l from  $10^6$  sporangiospores  $\text{ml}^{-1}$  suspension of the isolates were inoculated on the centre of the Petri-dish containing 20 ml culture media (0.5% peptone, 0.3% yeast extract, 1% agar) supplemented with 0.1% tributyrin (Sigma) (LIMA et al, 1991). After the inoculation, plates were incubated at 20 °C or 25 °C for 7 days.

For the induction of the lipase production in submerged culture,  $10^6$  sporangiospores  $\text{ml}^{-1}$  were inoculated into 30 ml minimal medium (0.15%  $(\text{NH}_4)_2\text{SO}_4$ , 0.15% Na-L-glutamate, 0.05% yeast nitrogen base) supplemented with 1% glucose, Tween 80, palm-, soybean-, sunflower-, olive-, extra virgin olive-, wheat germ-, corn germ-, sesame seed-, pumpkin seed- or cottonseed oil as sole carbon source and incubated under continuous shaking (200 rpm) at 25 °C for 12 days.

### Detection of the lipase activity

For sample preparation from submerged culture, 700  $\mu$ l of the filtrates were collected every second day and after filtration centrifuged at  $16.200 \times g$  for 30 min and the supernatant was stored at -20 °C. Enzyme activity was assayed by using *p*-nitrophenyl palmitate (Sigma, *p*NPP). Three mM concentration of *p*NPP stock solution was prepared in dimethyl sulfoxide (DMSO) and equal volume of potassium phosphate buffer (pH 6.8) was added. Fifty  $\mu$ l of buffered *p*NPP solution was given to 50  $\mu$ l diluted extract, and incubated for 30 min. at 25 °C. The reaction was stopped by 25  $\mu$ l of 0.1 M sodium carbonate, and the *p*-nitrophenol release was measured at 405 nm. One enzymatic unit was defined as the amount of enzyme that releases 1  $\mu$ mol of *p*-nitrophenol in 1 minute under the assay conditions. Enzyme activities were measured in 96-well microtiter plates using an ASYS Jupiter HD (ASYS Hitech) microplate reader. Enzyme activities were determined in three independent experiments.

## RESULTS

This work evaluated the extracellular lipase activity of 35 strains representing the Zygomycetes genera *Mortierella*, *Dissophora* and *Umbelopsis*. The investigated strains had been isolated from different soil samples (Table 1).

### Detection of lipase production in plates

The culturing media contained tributyrin to monitor the lipase activity. Incubation was performed at the optimal temperature conditions (20 °C or 25 °C) of each isolate. The level



of the lipase production was evaluated by measuring the diameter of the halo around the colonies that formed in consequence of the hydrolysis of tributyrin. The halo was measured in millimeters daily during the incubation period. The enzyme activity of the tested strains showed high variability and several isolates showing high activity were detected (Table 1). Growth of several isolates was fairly low on this media; unlike the small diameter of the colonies, the detected enzyme production was considerably high in some cases.

**Table 1. The investigated strains and the average diameter of halo representing the lipase activity of each isolate (best producers are highlighted with bold characters)**

Isolate	Code <sup>a</sup>	Source	Cultivation temperature (°C)	Diameter of halo (mm) <sup>b</sup>
<i>Dissophora ornata</i>	SZMC 11221	Forest soil/Columbia	25	5
<i>Mortierella gemmifera</i>	SZMC 11201	Pine forest soil/UK	20	1.5
<i>Mortierella longicollis</i>	SZMC 11208	Sandy soil/Australia	25	6
<i>Mortierella alpina</i>	SZMC 11213	Sandy soil/Australia	20	5
<i>Mortierella humilis</i>	SZMC 11220	Pine forest soil/Mexico	20	5
<i>Mortierella parvispora</i>	SZMC 11225	Soil/Germany	20	3
<i>Mortierella verticillata</i>	SZMC 11205	Tundra soil/USA	25	2
<i>Mortierella antarctica</i>	SZMC 11217	Soil, glacier/Antarctica	20	2
<i>Mortierella polygonia</i>	SZMC 11203	Soil/Netherlands	20	1
<i>Mortierella gamsii</i>	SZMC 11215	Soil/Netherlands	20	2
<i>Mortierella schmuckeri</i>	SZMC 11207	Soil (pH6.7)/Mexico	25	2
<i>Mortierella beljakovae</i>	SZMC 11232	Soil/Ukraine	20	2
<i>Mortierella camargensis</i>	SZMC 11227	Sandy soil/France	20	3
<i>Mortierella clonocystis</i>	SZMC 11238	Soil/Spain	20	3
<i>Mortierella cystojenkinii</i>	SZMC 11229	Agricultural soil/Netherlands	20	5
<i>Mortierella epicladia</i>	SZMC 11247	Soil/Spain	20	0.5
<i>Mortierella microzygospora</i>	SZMC 11248	Soil/Japan	20	3.5
<i>Mortierella minutissima</i> var. <i>dubia</i>	SZMC 11235	Soil/Germany	20	1.5
<i>Mortierella rostafinskii</i>	SZMC 11249	Soil/USA	20	0.5
<i>Mortierella verticillata</i>	SZMC 11233	Soil/France	20	1.5
<i>Mortierella verticillata</i>	SZMC 11236	Forest soil/China	20	2
<i>Mortierella verticillata</i>	SZMC 11230	Forest soil/Germany	20	2
<i>Umbelopsis angularis</i>	SZMC 11252	Soil/Netherlands	25	5
<i>Mortierella angusta</i>	SZMC 11254	Podzol soil/UK	20	3
<i>Mortierella exigua</i>	SZMC 11257	Soil/India	20	2
<i>Mortierella gamsii</i>	SZMC 11258	Forest soil/Germany	20	2
<i>Mortierella gamsii</i>	SZMC 11259	Forest soil/Germany	20	4
<i>Mortierella lignicola</i>	SZMC 11265	Soil/Germany	20	3
<i>Mortierella parvispora</i>	SZMC 11266	Soil/Germany	20	2.5
<i>Mortierella clausenii</i>	SZMC 11268	Soil/Switzerland	20	2
<i>Mortierella paraensis</i>	SZMC 11272	Rain forest soil/Brazil	20	3
<i>Mortierella rishiksha</i>	SZMC 11273	Forest soil/India	20	2
<i>Mortierella samyensis</i>	SZMC 11274	Soil/Ukraine	20	4
<i>Mortierella stylospora</i>	SZMC 11275	Sandy soil/Australia	20	1.5
<i>Mortierella globulifera</i>	SZMC 11260	Soil (pH6.4)/Germany	20	4

a: SZMC - Szeged Microbiological Collection

b: Values are measured on the seventh day of the cultivation.

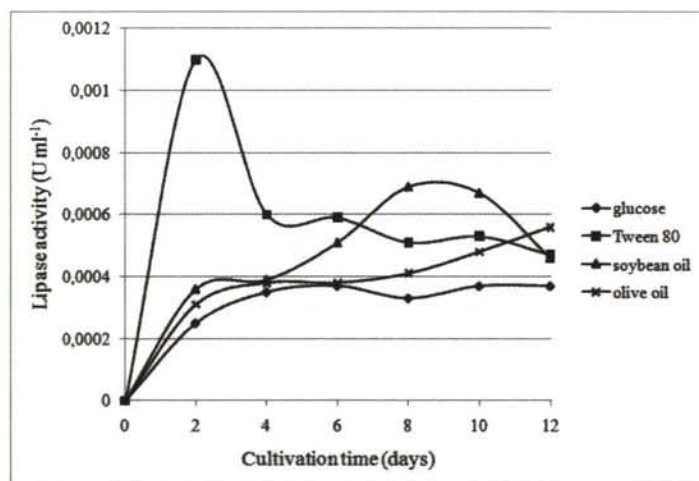
Based on the cultivation on plates, *Umbelopsis angularis* (SZMC 11252-Szeged Microbiological Collection), *Mortierella longicollis* (SZMC 11208), *M. alpina* (SZMC



11213), *M. humilis* (SZMC 11220), *M. cystojenkinii* (SZMC 11229) and *Dissophora ornata* (SZMC 11221) isolates showed the highest lipase production at the optimal cultivation temperature of each isolate (highlighted with bold characters in the Table 1). Significant enzyme production could also be observed by the *M. gamsii* (SZMC 11259), *M. sarnyensis* (SZMC 11274) and *M. globulifera* (SZMC 11260) strains at 20 °C (4 mm of halo). It is worth to mention that ALVES et al. (2002) presented the screening of *Mucor* strains isolated from herbivores dung and considered as good lipase producers. In the referenced work, the halo diameters were found between 4 and 6 mm at most of the isolates.

### Enzyme production in submerged cultures

*M. longicollis* was selected for further submerged culture studies to investigate the effects of different inductors (Tween 80, palm-, soybean-, sunflower-, olive-, extra virgin olive-, wheat germ-, corn germ-, sesame seed-, pumpkin seed- or cottonseed oil) on the enzyme activity. To evaluate the effect of lipid material, sporangiospores of the isolate were transferred to minimal medium supplemented with a given inductor and incubated at 25 °C for 12 days. Enzyme activities on each inductor were correlated to data obtained using 1% glucose as sole carbon source. Results show, that the lipase production was enhanced only by Tween 80, soybean- and olive oil, and the maximum level of the activity was on the second day by Tween 80, on the eighth day by soybean oil, and on the tenth day by olive oil (Figure 1). Similar stimulative effects of Tween 80 and different vegetable oils have been described for many bacterial and fungal lipases (SHARMA et al, 2001). No difference from the control enzyme activity was observed using palm-, sunflower-, extra virgin olive-, wheat germ-, corn germ-, sesame seed-, pumpkin seed- or cottonseed oil. Interestingly, CERTÍK et al. (1997) reported that sunflower oil is good substrate for *Mortierella* species; however, in our test, no significant lipase activity was detected using this substrate.



**Figure 1. Extracellular lipase production of *M. longicollis* in submerged fermentation using different carbon sources**

### CONCLUSIONS

Soil isolated zygomycetous fungi proved to be good sources of lipase enzymes. Besides other filamentous fungi isolated from soil (COSTA and PERALTA, 1999), the *D. ornata*, *M. longicollis* and *U. angularis* strains also have great potential to produce lipase enzymes into the environment. Different inductor oils to enhance the lipase production of *M. longicollis* were also investigated. It is proved that Tween 80, soybean- and olive oil are



good lipase inducers at this isolate; this result is similar to findings reported for some other filamentous fungi (SHARMA et al, 2001). Analysis and detection of lipases produced by other soil isolated *Mortierella* and *Umbelopsis* strains and testing of the enzyme activity on different oils and oil derivatives are in progress.

### ACKNOWLEDGEMENTS

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## **APPLICATION OF THE LoCoH METHOD IN THE ANALYSIS OF ROE DEER HABITAT USE**

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### **ABSTRACT**

The purposes of this study were (1) to demonstrate a novel and effective method of home range estimation, and (2) to determine the annual home range sizes of roe deer - captured and radio-tagged in a floodplain forest – with the fix-k LoCoH method. We used one year localization data of six roe deer equipped with GPS-GSM collars in January 2007. The home ranges were estimated with the fixed-k LoCoH (with 50% and 95% isopleths). To calculate the k value we used the “minimum spurious hole covering” (MSHC) rule. The obtained k values varied between 16 and 21. The size of the LoCoH home ranges varied between 230-540 hectares. The size of the 95% isopleths varied from 38 to 82 hectares, while the core areas (50% isopleths) were between only 5.02 and 8.23 hectares. To illustrate home ranges we also utilized the land cover map of the study area. Based on these results we propose this method to achieve better estimates of roe deer home ranges living in our study area.

**Keywords:** roe deer, home range, floodplain forest, LoCoH, k value, Hungary

### **INTRODUCTION**

Roe deer is one of the most important big game species for wildlife management in Hungary, occurring throughout the whole country (CSÁNYI ET AL., 2003; CSÁNYI ET AL., 2006A). To widen our knowledge about the habitat use and behaviour of European roe deer, the Institute for Wildlife Conservation (Szent István University) has been conducting a research programme in Jász-Nagykun-Szolnok county, Hungary since 2001 (CSÁNYI ET AL., 2003; CSÁNYI ET AL., 2006A,B). Up to the present to identify the home ranges we used minimum convex polygon (MCP) and Kernel home range (KHR) methods (WHITE AND GARROTT, 1990; SAMUEL AND FULLER, 1996; SEAMAN ET AL., 1999).

GETZ AND WILMERS (2004) described a new method for home range estimation: the local nearest-neighbour convex-hull (LoCoH), which was referred as a more accurate method (GETZ AND WILMERS, 2004; RYAN ET AL., 2006; RYAN, 2011). The purpose of our study is to identify the extent of the annual home ranges of roe deer living in a floodplain forest with this new method LoCoH.

Our questions were: (1) Is the LoCoH method better to determine the home ranges of roe deer? (2) What are the appropriate k values for the localization points of the captured and observed roe deer?

### **MATERIAL AND METHOD**

#### **The study area**

Roe deer were captured and radio-tracked on the area of Hofi Géza Hunting Club (game management unit). The game management unit has excellent brown hare and pheasant populations, as well as a quantitatively and qualitatively good roe deer population. The size

of the area is 5238 ha, with mostly agricultural fields (73.75%). Forest cover is only 6.56% in the study area – mainly floodplain forests of the Tisza River lying on the northern border of the area.

### **Capturing and marking**

Capturing and radio-tagging of roe deer took place on 17-18 January 2007 in the floodplain forest. In this paper we used the data of 6 roe deer (*Table 1*) which were supplied with GPS-GSM collars (GPS PRO Light-1 Collar). These collars are able to provide satellite localization and use GSM system for data transmission. The collars were made by the German Vectronic Aerospace GmbH ([www.vectronic.de](http://www.vectronic.de)).

### **Data collection with radio-telemetry**

The collars recorded localization points every three hours, which were stored on a SIM-card, and were sent in SMS format to the ground receiver through the GSM system. We then imported the localization information to the computer with Vectronic's own software. The numbers of localization points for each individual are showed in *Table 1*.

**Table 1. Data of studied roe deer and the number of their localizations in 2007**

Collar code	sex of individual	age at tagging	Number of localisation points (2007)
S1	female	2 years	2509
SG1	female	1 year	2521
B1	male	3 years	2520
B2	male	3 years	2521
S2	female	2 years	2504
S3	female	2 years	2488

### **Data processing and evaluation**

To visualize the localization data, and to calculate and represent the home ranges we used the ESRI ArcView GIS (Version 3.1) software. We determined the home ranges of individuals with the method of fixed-k LoCoH also known as k-NNCH (k - nearest neighbour convex hull). The fixed-k LoCoH takes every localisation point and locates its "k" nearest neighbours. It then forms a convex polygon hull using these points (much like the MCP approach, but for a subset of k points). Every hull successively is merged from small to large to form isopleths (contour lines). For example, the 50% isopleth contains 50% of the points (GETZ AND WILMERS, 2004; RYAN, 2011). (In our research we used 50% and 95% isopleths and we considered these areas as home ranges (95% isopleth) and core areas (50% isopleth) (MORSE ET AL., 2009). The k value was selected with the "minimum spurious hole covering" (MSHC) rule (GETZ AND WILMERS, 2004). In this study we represent the data of two males (B1, B2), three females (S1, S2, S3) and one non-adult female SG1) of the tagged animals (*Table 1*).

## **RESULTS**

### **Selecting the k value**

To select the value of k we used the MSHC rule. The home ranges at low k value contain a number of holes that disappear with the increasing value of k. If we know the topology of the area (inappropriate areas for habitat), we can select the smallest value of k with the



MSHC rule that produces a cover with the same topology as the given set (GETZ AND WILMERS, 2004). In our study area there are not any considerable inappropriate areas, so we could not decide what holes are spurious and what holes are real. Differences between real and spurious holes in LoCoH coverings of data sets should also be evident in plots of the number of holes in a particular LoCoH covering against the value of k: the covering of spurious holes should correspond to a levelling off of the resulting graph (GETZ AND WILMERS, 2004). The right k is the last value in the sequence before the sudden value increase (INTERNET 1). So we performed the calculation with k between 10 and 30 and we select the appropriate k from the graph. The “right” of final k is differed between the individuals, it varies from 16 to 21 (Table 2).

**Table 2. the sizes of the annual home ranges were estimated with different k values with the method locoh**

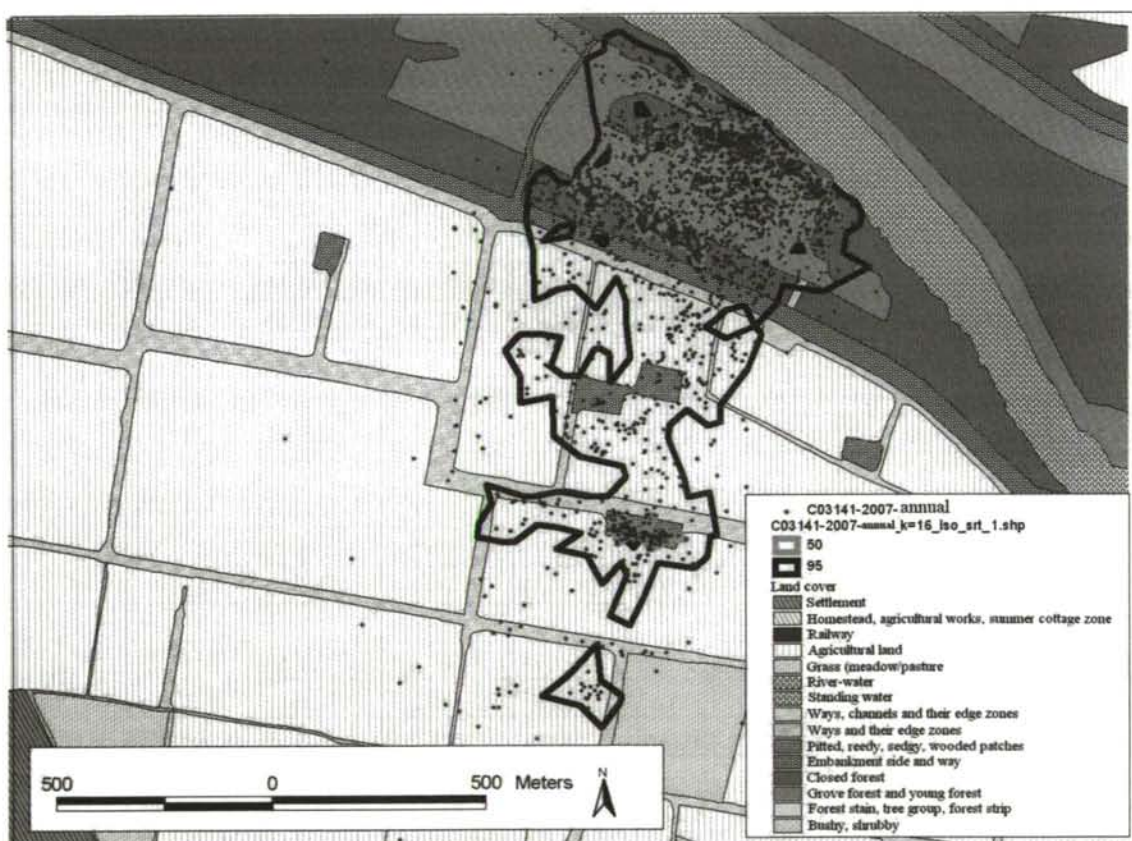
Collar code/ Value of k	Size of home range (100%, ha)					
	S1	SG1	B1	B2	S2	S3
10	302.31	272.84	243.09	492.76	249.13	116.30
11	305.36	310.33	249.23	520.68	262.04	175.75
12	337.76	317.57	254.52	527.40	268.46	186.85
13	343.18	324.58	269.70	529.08	281.09	196.55
14	345.69	331.46	274.49	531.70	287.02	200.55
15	347.69	335.74	276.15	534.56	290.56	217.36
16	350.25	337.14	277.75	536.71	294.43	224.60
17	354.40	340.57	282.53	538.21	295.53	227.45
18	358.43	341.71	282.89	539.35	298.63	228.71
19	358.96	344.22	283.34	553.25	300.91	231.86
20	360.93	346.03	286.46	604.72	304.32	232.39
21	363.03	346.55	287.18	606.02	306.01	233.60
22	366.87	350.47	287.26	608.08	308.72	233.86
23	367.58	351.02	287.52	609.86	309.81	236.67
24	370.53	351.31	287.73	618.18	311.06	237.11
25	373.16	352.33	287.75	669.08	312.69	237.53
26	375.23	352.68	288.83	669.23	316.18	237.99
27	375.37	355.70	290.12	670.57	318.59	238.24
28	376.25	356.16	290.16	671.25	319.25	239.25
29	376.31	356.48	290.18	673.05	320.37	239.71
30	376.38	356.99	290.20	673.05	321.79	239.11

#### The size of annual home ranges and core areas

The size of the annual LoCoH home ranges varied between 38-82 hectares (Table 3). The size of the core areas (50% isopleths) varied between 5-8.2 hectares. There is a sample map to demonstrate the fixed-k LoCoH home range and core area (Figure 1).

**Table 3: the sizes of each studied roe deer annual home ranges estimated with the LoCoH method**

Collar code	Value of k	Home range isopleths	
		95% (ha)	50% (ha)
S1	19	67.30	5.02
SG1	21	76.37	6.39
B1	16	54.02	6.01
B2	18	81.91	6.95
S2	17	72.86	8.23
S3	18	37.93	5.70

**Figure 1. The annual home range (95% isopleth, red) and core area (50% isopleth, pink) of the B1 roe deer**

## CONCLUSIONS

The data and maps are showing that the MSHC rule seems appropriate to select the k value as proposed by (GETZ AND WILMERS, 2004; MORSE ET AL., 2009), and can be used in further research. Based on our results the annual home ranges did not exceed 82 hectares, and the core areas did not attain nine hectares (!) in the case of the 6 individuals. This also means that the most important areas used by the tagged roe deer are merely some ten



hectares. We notice that there were considerable differences in the home range sizes between individuals.

It also can be seen that the localisation points have a number of centres (*Figure 1*). The LoCoH method is a good estimate if the localisation points are clustered around one or more centres of activity, because in such cases the minimum convex polygon will overestimate the area of the home ranges (RYAN, 2011).

Based on these results we suppose that this method may be better to estimate the home ranges of roe deer, but it needs more examination. We will compare the results of home range sizes to our earlier results of MCP and KHR home ranges methods.

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## USING DIFFERENT POTASSIUM AND MAGNESIUM TREATMENTS IN WATERMELON PRODUCTION

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### ABSTRACT

Since watermelon yield and quality are so greatly influenced by production practices, it is important that watermelon varieties be tested under adequate nutrition conditions. This study was conducted in 2009 and 2011 in county Békés. Watermelon cultivar *Crisby* was used in both year, furthermore in 2009 besides the non-grafted plants we used 2 grafted combinations, with the following rootstocks: *Strongtosa* (interspecific squash hybrid) and *Nun 3001* (*Lagenaria* type). Plants were grown under low tunnel conditions with intensive technology (soil covering, dripp-irragtion). 4 fertilization treatments were used with different potassium dose. In every treatment half of the plants got foliar magnesium fertilizer (EpsMicrotop), applied in concentration of 5%. The measurement of the yield and fruit weight (kg, average mass) were carried out in the field, while the fruit quality analyses (dry matter content, soluble solid content (Brix%), sugar content) were taken place in laboratory. In 2009 our results showed that interspecific rootstock gave higher yield and with higher potassium dozen the fruit quality won't be decreased. In 2011 the yield and TSS content was parallel with the increasing potassium doses. In both year the effect of the magnesium foliar fertilizer was more remarkable by lower potassium doses.

**Keywords:** Watermelon, potassium, grafted watermelon, yield, magnesium, foliar fertilizer

### INTRODUCTION

The watermelon is an important crop for growers in Hungary. It has the third biggest vegetable production area (cc. 6000 hectares) and a significant amount of the produced crop is exported.

Watermelon requires a certain amounts of nitrogen, phosphorous and potassium also. The nitrogen is essential for plant development and boosts both plant growth and crop yield. Adequate K nutrition has been associated with increased yields, fruit size, increased soluble solids and ascorbic acid concentrations, improved fruit colour, increased shelf life, and shipping quality of many horticultural crops. In order to obtain high yield and good quality of watermelon, there is need to augment the nutrient status of the soil to meet the crop's need and thereby maintaining the fertility of the soil (NAGY, 2005).

Many changes have occurred in watermelon production practices in the last few years, including new cultural systems such as polyethylene mulch and drip irrigation changes in cultivars and using grafted plants. Seed companies are currently experimenting with grafted plants under Hungarian conditions.

The fact that certain rootstocks show stronger resistance to soil pathogens, greater tolerance of low soil temperatures, as well as of salt stress has been well documented in the past (DAVIS ET AL., 2008; HOYOS, 2001; ODA, 2007). Thus, the selection of rootstocks is rarely based on characteristics related to nutrient uptake, but rather almost always on resistance to environmental stress (RUIZ ET AL., 1997). Knowledge of the rootstock/scion nutritional relationship could be decisive in choosing rootstocks tolerant or resistant to soils that are deficient or toxic in one or more nutrients, as well as in preparing fertilization



programmes after the grafted plant is transplanted (CHAPLIN AND WESTWOOD, 1980). Some researcher reported that quality (Brix, firmness, rind thickness etc.) of watermelon was greatly affected by which rootstock was used, but it can also be influenced by the nutrient dosage (GAO AND LIAO, 2006; LEE AND ODA, 2003; MASUDA ET AL., 1986; YAMASAKI ET AL., 1994). Since watermelon yield and quality are so greatly influenced by production practices, it is important that watermelon varieties be tested under adequate nutrition conditions.

## MATERIAL AND METHOD

### Plant material and culture conditions

In both experimental year (2009 and 2011) the used cultivar was: *Crisby* (seed source: Nunhems) In 2009 besides the non-grafted plants we used 2 grafted combinations with the following rootstocks: *Cucurbita maxima* x *Cucurbita moschata* interspecific (squash) hybrid: *Strongtosa* (seed source: Syngenta), and a *Lagenaria* type (bottle gourd): *Nun 3001* (seed source: Nunhems).

In 2009 the experiment was carried out in Békés county in Dombegyház, while in 2011 the experiment was set up in Kunágota. In both area the soil type is chernozem. Before planting soil analysis was carried out. The results showed that both area are very good provided in potassium and magnesium. Carbonate and mold content were also very high. The experiment was conducted in growers' farm using intensive technology (drip irrigation, plastic soil cover, low plastic tunnel covering). In 2009 plants were transplanted to the field on 100 cm spacing within the row and 320 cm between rows (3125 per ha). This arrangement is adequate for grafted plants, but for non-grafted plants the proposed plant density is 7000 per hectare or more. In spite of this we had to use equal spacing because of the arrangement of the irrigation system. The experiment was set up with 448 plant in total. In 2011 non-grafted plants were used only with a spacing 60 cm within the row and 170 cm between the rows (9804 plant/ha). In this year 1800 plants were planted.

Both year the planting was took place on 15th April.

In 2009, when grafted plants were also studied the applied fertilizer dose was calculated on the basis on average yield 90 t ha<sup>-1</sup>. In 2011, when the experiment included non-grafted plants only it was calculated on average yield 50 t ha<sup>-1</sup>. On the score of potassium there were 4 different treatment and a control treatment. In **2009** the control treatment did not contain fertilizers at all. The following fertilizer doses were applied in the 4 treatment: N:145 kg ha<sup>-1</sup>, P<sub>2</sub>O<sub>5</sub>: 65 kg ha<sup>-1</sup> by all treatments, K<sub>2</sub>O: **1. treatment** 0 kg ha<sup>-1</sup>, **2. treatment** 160 kg ha<sup>-1</sup>, **3. treatment**: 325 kg ha<sup>-1</sup>, **4. treatment**: 485 kg ha<sup>-1</sup>.

In **2011** the growers fertigation technology (N: 90 kg ha<sup>-1</sup>, P<sub>2</sub>O<sub>5</sub>: 28 kg ha<sup>-1</sup>, K<sub>2</sub>O: 50 kg ha<sup>-1</sup>) was used as a control to avoid yield loss. The fertilizer doses in the 4 treatment were: N:80 kg ha<sup>-1</sup>, P<sub>2</sub>O<sub>5</sub>: 36 kg ha<sup>-1</sup> by all treatments, K<sub>2</sub>O: **1. treatment**: 0 kg ha<sup>-1</sup>, **2. treatment**: 90 kg ha<sup>-1</sup>, **3. treatment**:180 kg ha<sup>-1</sup>, **4. treatment**: 270 kg ha<sup>-1</sup>.

One treatment was one row because of the build up of the irrigation system. The drip lines for each row were equipped with a valve at the header line. This allowed water/fertilizer to be directed to selected rows during application.

The total amount of the active substances were given divided in different development phase (radication, intensive growth, first female flowers, small melons, ½ size crops).

In every treatment half of the plants got foliar magnesium fertilizer (EpsMicrotop 15% MgO12%+S12%+B1%+Mn1%). These fertilizer mixtures were diluted with water and applied 5% concentration. In 2009 the number of the application varied with the treatments. The 1st treatment were sprayed 4 times (15kg ha<sup>-1</sup>), the 2nd treatment 3 times



(11.25 kg ha<sup>-1</sup>), the 3rd treatment 2 times (7.5 kg ha<sup>-1</sup> and the 4th treatment 1time (3.75 kg ha<sup>-1</sup>). In 2011 plant were treated with foliar fertilizer three times in each treatment (MgO 11.25 kg ha<sup>-1</sup>), started with initial fruit set. All foliar applications were made with a backpack sprayer and hand boom equipped with flat fan nozzles.

Fungicides and insecticides were applied separately, but in a similar manner as needed throughout the season. For evaluation the treatments we collected the fruits in every row in four replication.

### Measurements:

The measurement of the yield and fruit weight (kg, average mass) was carried out in the field, while the fruit quality analyses were taken place in laboratory. These contained the following measurements: (1) dry matter (%) (husk+fruitflesh and just fruitflesh), (2) soluble solid content (Brix%) (fruitflesh), (3) sugar content (%) (fruitflesh): a) total sugar content (%), b) glucose+fructose content (%), c) sucrose content (%)

**Table 1. The effect of K doses and EpsoMicrotop foliar fertilization on the yield of watermelon in 2009**

Treatment (K <sub>2</sub> O kg ha <sup>-1</sup> )	Yield (kg m <sup>-2</sup> )			Increase (%)			The effect from EpsoMicrotop on the yield on the given K <sub>2</sub> O doses (%)		
	non-grafted	Crisby/ Strongto sa	Crisby/ Nun 3001	non-grafted	Crisby/ Strongto sa	Crisby/ Nun 3001	non-grafted	Crisby/ Strongto sa	Crisby/ Nun300 1
Controll	2.25	2.3	2.25	100	100	100			
1. (0)	2.7	7.40	7.32	120	329	325			
2. (160)	4.12	7.36	6.78	183	327	301			
3. (325)	2.6	8.45	7.18	116	376	319			
4. (485)	3.16	9.28	9.37	140	412	416			
1.(0)+ EpsoMicrotop	2.48	8.93	7.83	100	100	100	92	121	107
2. (160)+ EpsoMicrotop	3.47	8.24	6.33	140	92	81	84	112	93
3. (325)+ EpsoMicrotop	2.31	8.46	7.42	93	95	95	89	100	103
4. (485)+ EpsoMicrotop	3.26	9.24	9.37	131	103	120	103	100	100

## RESULTS

### Quantitative parameters

In 2009 fruit were obtained from multiple harvests, started with the non-grafted plants in the second half of June. Last harvest date was on 15th July. From the grafted plants we could harvest longer than from the non-grafted ones (*Table 1*). In 2011 the harvest was begun in mid-June and continued until early August. The harvested fruit were numbered and measured in the field and some fruit were transported to the University's laboratory for qualitative analysis.

In 2009 the grafted and the non-grafted plants were planted with equal spacing because of the arrangement of the irrigation system. It follows that some parameters are not comparable among grafted and non-grafted plants.

The very big difference in the yield of grafted and non-grafted plants was due to plant density only favourable to grafted plants. The yield showed positive correlation with the potassium dose by grafted 'Crisby' on the rootstock *Strongtosa*. The foliar fertilization has



higher effect by lower potassium dose. In total yield the interspecific squash rootstock produced a higher yield.

Connection between the yield and the treatments could not be found by grafted 'Crisby' on rootstock *Nun 3001*. Though the foliar fertilization gave significant higher yield by the highest potassium dose.

In 2011 the highest yield was obtained from the higher potassium dose (180 and 270 kg K<sub>2</sub>O ha<sup>-1</sup>). The applied doses gave significant differences compared to the control (the grower's technology), the enhanced dose increased the yield with 18% and 20% (Table 2.). Magnesium, given as a foliar fertilizer, had similar effect on the yield. The last column from Table 2. shows the effect from Epsomicrotop. The effect was more remarkable by lower potassium doses. It is notable that by 0 kg K<sub>2</sub>O ha<sup>-1</sup> the increase was 15%.

Evaluate the results from fruit weight we found the same results, the only difference was that by 270 kg K<sub>2</sub>O ha<sup>-1</sup> doses the fruit size decreased but not significantly.

**Table 2. The effect of K doses and Epsomicrotop foliar fertilization on the yield of watermelon in 2011**

Treatment (K <sub>2</sub> O kg ha <sup>-1</sup> )	Yield (kg m <sup>-2</sup> )	Increase (%)	+ Epsomicrotop yield kg m <sup>-2</sup>	Increase (%)	The effect from Epsomicrotop on the yield on the given K <sub>2</sub> O doses (%)
Control (50)	5.45	100	5.68	100	104.2
1. (0)	4.21	77	4.84	85	115
2. (90)	5.83	107	6.02	106	103
3. (180)	6.42	118	6.48	114	100.9
4. (270)	6.57	120.5	6.65	117	101.2

### Qualitative parameters

For determination of total soluble solids (TSS) digital refractometer were used.

Lower fruit quality issues reported for watermelon due to grafting, include reduced soluble solids content, insipid taste, poor texture (TRAKA-MAVRONA ET AL., 2000) Our results were parallel, in the non-grafted fruits higher soluble solid content were measured. TSS ranged between 10,6 and 12,5 Brix%.

Using the two different rootstock type, significant difference were observed. The mature fruit from the *Crisby/Nun3001* combination had significantly lower soluble solid contents compared to that from *Crisby/Strongotsa* seedlings. The higher potassium dose resulted higher TSS content, and Epsomicrotop treatments improved it even more.

In 2011 measuring the total soluble solids (TSS) in the fruits, significant correlations were found between the values and the applied fertigation. The values increased parallel with the increasing doses of potassium. The effect from Epsomicrotop was remarkable by 0 kg K<sub>2</sub>O ha<sup>-1</sup>.

The total sugar content (%) and the different sugar fractions were measured from the fruit flesh. Glucose, fructose and sucrose content was dissociated.

Concerning non-grafted watermelon the sugar content showed correlation with the potassium dose in both years. In case of the two types of rootstock the results were similar to the total soluble solid content. The *Lagenaria* rootstock resulted lower sugar content compared to interspecific squash rootstock and non-grafted plants. But higher potassium dose increased the sugar content significantly (from 3.7% to 6.9%) by enhancing the sucrose content (from 0.2% to 4.3%). The foliar fertilization revised the sugar content even more in all treatments, both years.



## CONCLUSIONS

In the first experiment (2009) grafted plants were also studied besides non-grafted *Crisby*. Both rootstock-scion combination resulted higher yield compared to non-grafted plants. The effect of potassium was more conspicuous by the interspecific squash rootstock (*Strongtosa*). By grafted plants the foliar fertilization with magnesium has also good effect on the yield. In 2009 regarding the non-grafted *Crisby* the lower potassium dose (treatment 2) resulted the highest yield, while in 2011 the yield was parallel with the potassium dose. The highest potassium dose enhanced the yield by 20% compared to control (grower's technology).

EpsomMicrotop as a magnesium foliar fertilizer showed also positive results, especially by reduced potassium dose.

Sugar content appears to be one of the most important characteristics of a good-quality watermelon, based on the fruit quality indices routinely measured by scientists. Some previous research has generally shown that grafting has negative effect on TSS content in watermelon fruit when grafting watermelon onto *C. maxima* x *C. moschata*, or *L. siceraria* rootstocks (IOANNOU ET AL., 2002). In our experiment we got similar results, but with higher potassium dose we could reduce the decrease. Regarding the non-grafted plants the increased potassium dose resulted higher sugar content in both years. The magnesium revised the sugar content of the fruits in every treatment, especially besides lower potassium dose.

Considering the opportunities for grower's, among the many plant mineral nutrients, potassium and magnesium stands out as a cautions having very strong influence on quality attributes that determine fruit marketability.

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## **ECONOMIC BENEFITS ARISING FROM THE PREBIOTICALLY ENHANCED SUPPLEMENTARY FEEDING OF HONEYBEE COLONIES**

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### **ABSTRACT**

Addition of prebiotic (acidifying) products to the supplementary feeds provided to honeybee colonies has a positive effect on colony health through favouring the development of a beneficial intestinal microflora. It also increases queen fecundity and oviposition leading to fast colony recruitment. We report the economic benefits of using acidifying substances (cider vinegar and lactic acid) in spring feeding supplements given to colonies. Trialling was conducted at Jebel, Romania between April 15 and May 4 2011 using 30 colonies which each received 1.4 l per week of supplemental feed sugar syrup with these substances added. Acacia honey production was assessed by weight at the end of May. Production was found to be significantly higher in the treatment group compared with the control ( $p < 0.05$ ) resulting, for the treatment group, in a predicted economic premium ranging between 9.38 and 22.06%.

**Keywords:** economic benefit, prebiotic products, honeybee colonies

### **INTRODUCTION**

In order to maintain colonies in a biologically strong and productive condition the practice of beekeeping requires not only the availability of sufficient nectar and pollen resources during the entire foraging period but also the feeding of the bees during periods when natural resources do not suffice (BURA AND PĂTRUICĂ, 2003). Beekeepers' priorities are the maintenance of colony health and the provision of adequate pollen and nectar sources for their stocks.

The normal practice during periods of nectar shortage, if the beekeeper does not have a reserve of filled combs, is to provide a supplementary feed of sugar syrup (CHIRILĂ AND PĂTRUICĂ, 2005; MORARU, 2006).

Many researchers have reported benefits to colony health and development resulting from the incorporation of plant extracts in the supplementary syrup feeds. (BURA ET AL., 2004; PĂTRUICĂ ET AL., 2006; TOFALVI, 2009; PĂTRUICĂ ET AL., 2011).

POPOVICI (2011) found that addition of citric acid to protein-energy bee candy increased brood production in comparison with the control group. Similar results were found by PĂTRUICĂ ET AL. (2011<sub>a, b</sub>) who doped sugar syrups with different dosages of lactic and acetic acids and cider vinegar.

This investigation aimed to establish whether, alongside these beneficial consequences for bee health, the addition of acidifiers (specifically lactic acid and cider vinegar) resulted in cost-effective economic benefits through greater honey production.



## MATERIALS AND METHODS

30 colonies of honeybees (*Apis mellifera carpatica*) housed in multisection hives were studied, divided into three equal treatment groups of 10 colonies of comparable vigour and with queens of the same age.

Trialling was conducted at Jebel, Romania between April 15 and May 4 2011, with the colonies being fed sugar syrup incorporating, where appropriate, acidifiers (lactic acid or cider vinegar). Feeds were made up as shown in *Table 1*.

**Table 1. Treatment regimes**

No	Experimental variants	Food structure			
		Sugar syrup (ml)	pH of syrup	98% Lactic acid (ml)	Cider vinegar (ml)
1.	Control group (M)	1000	6.5	-	-
2.	Experimental group 1 (LE <sub>1</sub> )	1000	4.2	1.5	
3.	Experimental group 2 (LE <sub>2</sub> )	1000	5	-	12.5

Each colony was given, in feeders, a weekly supplement of 1.4 l syrup (1:1 sugar:water, 1kg sugar to 1 litre water) acidified as shown above. On May 10 the colonies were relocated for them to forage in the vicinity of rural acacia plantations. All colonies were maintained in multisection hives and given the same foraging opportunities. Statistical analysis was performed using the MINITAB 14 software package.

## RESULTS

Honey production per colony is dependent on a number of factors: colony strength and health, the availability of nectar within bee flight range radius and meteorological factors (temperature, humidity and wind).

Evaluation of the effect of acidification of feed supplement on honey production was performed by weighing the quantities of honey accumulated by the colonies under study. This was done at the end of May.

Colonies fed syrup supplements acidified with lactic acid or cider vinegar produced between 15.5 kg and 19.1 kg of acacia honey (*table 2*).

**Table 2. Production of acacia honey, statistical analysis**

No	Experimental variants	Statistical parameters					Statistical significance
		n	$\bar{x}$	$S\bar{x}$	S	CV	
1.	Control group (M)	10	15.5	±1.87	3.25	20.07	-
2.	Experimental group 1 (LE <sub>1</sub> )	10	19.1	±2.32	5.04	18.98	*
3.	Experimental group 2 (LE <sub>2</sub> )	10	16.9	±2.08	5.36	17.33	is

\*p<0.05

is - insignificant

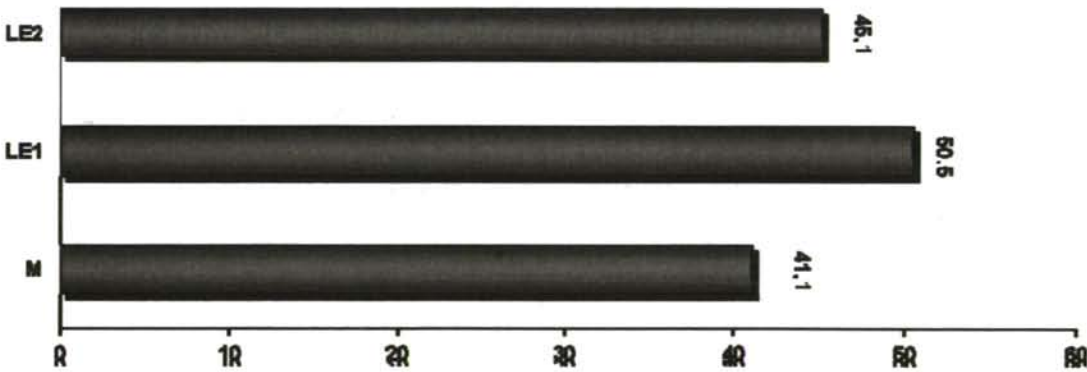
The data presented in table 2 show that the colonies fed with acidified syrup (lactic acid or cider vinegar) showed levels of honey production between 9.03% and 23.33% greater than the control group. It has been shown that acidification to a level of pH 4.2 had the best effect on colony development, these colonies producing between 900 and 5690 more brood

cells than experimental treatments fed syrup with a pH of 5-6.5 (PĂTRUICĂ ET AL., 2011<sub>a</sub>). We can say that the feeding of colonies with the acidified syrups described stimulated egg deposition by the queen, thus allowing the colonies concerned to exploit the available acacia nectar better since they were able to deploy a larger number of foraging workers. Supplemental feed costs for the bee colonies over three weeks ranged between €2.9 and €4.3 per colony. The cost of prophylactic treatment against *Varroa destructor* mite was €1 per colony for all colonies studied and the cost of transport to the foraging area was €1.5 per colony. The labour, carried out by the beekeeper's family, involved an average of two hours per day. Acacia honey was sold for €3/kg.

**Table 3. Economic benefits of the use of prebiotic products in the supplemental feed of bee colonies**

No.	Specification	Experimental variants		
		Control group (M)	Experimental group 1 (LE <sub>1</sub> )	Experimental group 2 (LE <sub>2</sub> )
1	Cost of food supplement (€/colony)	2.90	4.30	3.10
2	Cost of treatment (€/colony)	1	1	1
3	Cost of transport to field (€/colony)	1.5	1.5	1.5
4	Total cost (€/colony)	5.4	6.8	5.6
5	Income (€/colony)	46.50	57.30	50.7
6	Profit (€/colony)	41.1	50.5	45.1
7	Profit (€/study group (10 colonies)	411	505	451

The use of acidifiers (lactic acid or cider vinegar) in the concentrations studied led to the obtaining of a profit 9.73% greater than the control group for the group fed syrup treated with cider vinegar, with a 22.87% enhancement of profit being registered for the group fed syrup treated with 1.5 ml 98% lactic acid (figure 1).



**Figure 1 Profit/colony following feeding with acidified syrup supplements**

**CONCLUSIONS**

1. The addition of acidifiers (lactic acid or cider vinegar) to supplementary feeds resulted in better colony growth (12.64% and 15.34% more brood production respectively, compared with the unacidified control). By the time the acacia nectar was ready for collecting these had developed into foraging workers.



2. Proceeds from the sale of the first take of honey were between 9.03% and 23.22% greater for colonies which had received acidified feed, with the best profit shown from colonies whose sugar syrup feed had been dosed with 1.5 ml lactic acid.
3. Total maintenance expenses for all the colonies studied were between 5.4 €/colony (LM) and 6.8 €/colony (LE<sub>1</sub>), these costs including feed costs, two prophylactic treatments against *Varroa destructor* mites and colony transport costs to the foraging area.
4. The profit obtained following lactic acid or cider vinegar dosing of supplementary feed syrup was greater by between 9.73% and 22.87%. This allows us to recommend such treatment in the early spring after the cleaning flight.

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## **THE EFFECTS OF USING CHICKEN GRILL OIL INSTEAD OF THE SUNFLOWER OIL ON PERFORMANCE, BLOOD PARAMETERS, CHOLESTEROL AND FATTY ACID COMPOSITION OF EGG YOLK IN LAYING JAPANESE QUAIL (*COTURNIX COTURNIX JAPONICA*)**

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### **ABSTRACT**

This study was carried out to determine the effect of using chicken grill oil instead of the sunflower oil in laying Japanese quail diets on egg production, feed intake, feed conversion ratio, egg quality, blood parameters, cholesterol and fatty acid composition of egg yolk. A total of 192, thirteen week old Japanese quail were allocated to four groups with six replicates containing eight quail each. The diets were isonitrogenous and isocaloric and included 20 % crude protein and 2900 kcal/kg ME. The diets contained 5% sunflower oil (SO1), 5% chicken grill oil (CO1), 7.5% sunflower oil (SO2), 7.5% chicken grill oil (CO2). Experimental diets and water were provided *ad libitum* throughout the 9 weeks. There were no significant differences in initial body weight among groups, but there were highly significant ( $p<0.001$ ) differences in final body weight among groups: 296.62, 286.62, 308.24, 276.35 g for SO1, CO1, SO2 and CO2 respectively. Higher egg production ( $p<0.001$ ) was obtained for quail fed the CO1 and the CO2 diet (5% and 7.5% chicken grill oil respectively) than for quail fed the SO1 or the SO2 (5% and 7.5% sunflower oil). The cholesterol content in blood serum (195.61, 197.12, 157.37 and 176.49 mg/dl respectively) was not statistically significant. At the end of the study cholesterol content of the egg yolk (49.70, 49.54, 45.55 and 44.06 mg/g egg yolk respectively) was lowest in ( $p<0.05$ ) the laying quail fed the CO2 diet. All blood parameters including haemoglobin, haematocrit, serum cholesterol and serum glucose were not affected by diets except serum triglyceride and serum protein ( $p<0.01$ ). Dietary chicken grill oil had no adverse effect on egg weight, cholesterol and fatty acid composition of the egg yolk and feed conversion ratio. Therefore, chicken grill oil can be use up to 7.5% of the laying quail diets.

**Key words:** blood parameter, egg, oil, performance, quail,

## **INTRODUCTION**

Metabolic rates and metabolic energy requirements of poultry are very high. Therefore poultry diets should be rich in energy. Oils are important in the diet of poultry as concentrated sources of energy. Use of fats for animal feed has many advantages such as source of energy, increase growth rates, increase feed efficiency, source of essential fatty acid, increase palatability of feeds and lower heat increment during heat stress which keeps caloric intake up etc. (REID, 1985; SENKOYLU, 1990; OZDOĞAN AND SARI, 2001). Because of the high prices, oils being used as a supplement and increases the total cost of diets. Therefore cheaper alternative of by-products from oil industry have been started to be use (CETINGUL and INAL, 2009). The objective of this study was to investigate the effects of using chicken grill oil instead of sunflower oil on feed conversion ratio, cholesterol and fatty acid composition of egg yolk, egg weight of laying quail. Further, the study should contribute to the reduction of environmental pollution.



## MATERIALS AND METHODS

In this study a total of 192, thirteen week old Japanese quail were divided into four groups with six replicates containing eight quail each. The diets were isonitrogenous and isocaloric and included 20 % crude protein and 2900 kcal/kg ME. Diets contained 5% sunflower oil (SO1), 5% chicken grill oil (CO1), 7.5% sunflower oil (SO2), 7.5% chicken grill oil (CO2). Firstly, in this study a local ethics committee report was taken. Experimental diets and water were provided ad libitum throughout the nine weeks.

The nutrient compositions of diets were determined according to the AOAC (1984). The analysis of serum and egg yolk total cholesterol and triglyceride were measured on spectrophotometer (Shimatzu UV-1601 Model) by using commercially available kits. During the experiment, egg yolk cholesterol analysis was performed 3 times. Serum protein and glucose levels were estimated by the method of the Biuret (Karagul et al., 2000) and Feteris (1965) respectively. Haemoglobin (Tietz, 1987) and haematocrit were also determined. Fatty acid profiles of experimental fats and egg yolks were determined using QP 5050 GC /MS.

Statistical analyses of data were performed by computer. Differences between obtained values were carried out by analysis of variance (ANOVA) and the significance of mean differences was tested by the Duncan's test (Ozdamar, 1997).

## RESULTS AND DISCUSSION

In this study, there were no significant differences in initial body weight among the groups, but there were highly significant ( $p < 0.001$ ) differences in final body weight among the groups: 296.62, 286.62, 308.24, 276.35 g for SO, CO1, SO2 and CO2 respectively. On the other hand, according to ATAKISI *et al.* (2009), Omega- 3 fatty acids had no effects on the body weights, or egg and egg yolk weights. In our study, a decrease has been observed in the egg weight at the end of the experiment with CO2 (7.5% chicken grill oil) diets according to CO1, SO1 and SO2 (sunflower oil) diets ( $p < 0.05$ ) (Table 1).

**Table 1. Cholesterol levels (mg/g) in egg yolk of quail fed diets with different amounts sunflower oil and chicken grill oil.**

Items	Groups				P-value
	SO1	CO1	SO2	CO2	
Egg weigh (1), g	13,8848	13,3484	13,8478	13,4484	0.392
Boiled egg yolk weight (1), g	4,2872 <sup>ab</sup>	4,0808 <sup>ab</sup>	4,3972 <sup>b</sup>	4,0113 <sup>a</sup>	0,063
Egg yolk cholesterol (1), mg/g	39,6058 <sup>ab</sup>	38,2708 <sup>a</sup>	39,9000 <sup>ab</sup>	41,3892 <sup>b</sup>	0,144
Egg weigh (2), g	12,9737 <sup>a</sup>	13,1628 <sup>a</sup>	14,1508 <sup>b</sup>	13,3680 <sup>a</sup>	0.013
Boiled egg yolk weight (2), g	4,1570	4,3372	4,5855	4,5494	0.337
Egg yolk cholesterol (2), mg/g	44,6533	44,1717	44,9317	45,1783	0.917
Egg weigh (3), g	13,5771 <sup>b</sup>	13,3431 <sup>ab</sup>	12,9227 <sup>ab</sup>	12,7033 <sup>a</sup>	0.048
Boiled egg yolk weight (3), g	4,4290 <sup>b</sup>	4,2327 <sup>b</sup>	4,2641 <sup>b</sup>	3,9007 <sup>a</sup>	0.016
Egg yolk cholesterol (3), mg/g	49,7042 <sup>b</sup>	49,5383 <sup>b</sup>	45,5450 <sup>ab</sup>	44,0550 <sup>a</sup>	0.016
p<0.05, Different superscripts <sup>a,b</sup> in the same row indicate significant differences between groups. 5% sunflower oil (SO1), 5% chicken grill oil (CO1), 7.5% sunflower oil (SO2), 7.5% chicken grill oil (CO2)					



Higher egg production ( $p<0.001$ ) was obtained for the quail fed CO1 and CO2 diet (5% and 7.5% chicken grill oil respectively) than for the quail fed the SO1 and SO2 (5% and 7.5% sunflower oil) (Table 2). Contrary to our results CELEBI AND UTLU (2006) reported that the highest egg production was obtained from sunflower oil containing high level of linoleic acid. The highest FCR1 and FCR2 (feed conversion ratio) were obtained from SO2 (7.5% sunflower oil). The addition of 5% and 7.5% chicken grill oil has positive effect on FCR compared with other groups (5% and 7.5% sunflower oil). SHAHRYAR ET AL. (2011) reported that the addition of 3% canola oil or 3% poultry fat has positive effect on feed conversion compared with other groups.

<b>Table 2. The effects of sunflower oil and chicken grill oil on performance of laying</b>					
Items	Groups				P-value
	SO1	CO1	SO2	CO2	
Feed intake (g feed/quail/day)	40.61 <sup>b</sup>	42.76 <sup>c</sup>	40.64 <sup>b</sup>	38.75 <sup>a</sup>	<0.001
FCR1* (g, feed/dozen egg)	566.05a	555.98a	680.22b	528.20a	<0.001
FCR2 (kg feed/kg egg)	3.44a	3.28a	4.29b	3.30a	<0.001
Egg production, %	87.15 <sup>b</sup>	92.91 <sup>c</sup>	73.42 <sup>a</sup>	90.26 <sup>bc</sup>	<0.001

\*FCR; Feed Conversio Ratio, \*\*\*  $p<0.001$ , 5% sunflower oil (SO1), 5% chicken grill oil (CO1), 7.5% sunflower oil (SO2), 7.5% chicken grill oil (CO2). Different superscripts <sup>a,b,c</sup> in the same row indicate significant differences between groups.

The cholesterol content in the blood serum (195.61, 197.12, 157.37 and 176.49 mg/dl for SO1, CO1, SO2 and CO2 respectively) was not statistically significant. However, the findings of this experiment do not agree with those found by CELEBI AND UTLU (2006) who observed a reduction of serum total cholesterol when quail were fed diets with different oil (119.17, 138.66, 136.67, 86.50, 82.67 mg/dl for control, tallow oil, a mixture of tallow and flaxseed oil, sunflower oil and flaxseed oil, respectively, ( $p<0.05$ )). This findings are different from QURESHI ET AL. (2004), who reported the serum cholesterol values were significantly higher ( $p<0.05$ ) in chickens fed animal fat than fed vegetable fat. On the other hand, at the end of the study cholesterol content of yolk (49.70, 49.54, 45.55 and 44.06 mg/g egg yolk for SO1, CO1, SO2 and CO2 respectively) was lower ( $p<0.05$ ) in laying quail fed the CO2 diet (7.5%chicken grill oil) (Table 1). FILARDI ET AL. (2005) reported that the concentrations of saturated, monounsaturated, and PUFA in the egg yolks were significantly affected by the addition of different fat sources to diets. MAZALLI ET AL. (2004) reported that cholesterol content in eggs was significantly ( $p<0.05$ ) higher for hens fed the control diet than for hens fed the various oil treatments.

Blood parameters including haemoglobin, haematocrit, serum cholesterol, serum glucose were not affected by diets except for serum triglyceride and serum protein  $p<0.01$ ) (Table 3). Similarly GROBAS ET AL. (2001) reported positive effects from the intake of monounsaturated fatty acid on health, with reduce triglyceride concentration in blood. But ATAKISI ET AL. (2009) reported that omega-3 fatty acids reduced egg and plasma cholesterol as well as plasma glucose level and no change was observed in triglyceride levels with the supplementation in quails. Similar to our results, PAL ET AL. (2002) reported that the type of fat added to the diet did not affect the glucose levels and omega-3 fatty acids changed the effects of insulin and glucagon on the plasma glucose.



**Table 3. The effects of sunflower oil and chicken grill oil on blood parameters of laying quails**

	Group				P-value
	SO1	CO1	SO2	CO2	
Haemoglobin, g/dl	21,2117	21,9400	18,1867	20,2250	0,094
Haematocrit, %	37,8333	39,3333	41,3333	39,1667	0,164
Serum Cholesterol, mg/dl	195,6100	197,1283	157,3667	176,4850	0,073
Serum TG, mg/dl	831,1600 <sup>b</sup>	820,3783 <sup>b</sup>	519,1300 <sup>a</sup>	695,5917 <sup>b</sup>	0.002
Serum glucose, mg/dl	220,7100	217,9567	259,0583	224,5917	0.280
Serum protein, g/dl	4,4033 <sup>b</sup>	4,4350 <sup>b</sup>	3,3000 <sup>a</sup>	4,1867 <sup>b</sup>	0.002

p<0.01, 5% sunflower oil (SO1), 5% chicken grill oil (CO1), 7.5% sunflower oil (SO2), 7.5% chicken grill oil (CO2). Different superscripts <sup>a,b</sup> in the same row indicate significant differences between groups

Table 4 shows the effects of dietary fats on yolk fatty acid composition. The stearic acid in egg yolk for quails fed the SO1 diet was higher than those fed the diets (CO1, SO2, and CO2). CEYLAN ET AL. (2011) reported fatty acids profile of the egg yolk was significantly altered by type and level of dietary fat, but cholesterol content of yolk was unchanged. JIANG *et al.* (1991) reported that the high linoleic acid content of sunflower seed diet increased the level of stearic acid in yolk. Similarly, CELEBI AND MACIT, (2008) reported that the fatty acid composition of egg yolk lipids were significantly affected by dietary fatty acid composition. In this study, the fatty acid content of chicken grill oil is 0.46% C14:0, 20.29% C16:0, 1.95% C16:1, 6.63% C18:0, 30.63% C18:1, 1.68% C18:1(n-7), 35.11% C18:2, 2.81% C18:3. FILARDI ET AL. (2005) also reported that there was no effect of the fat sources on the concentration of n-3 fatty acids in the yolk. But a significant effect was observed on the level of n-6 fatty acids with the lowest concentration determined by the addition of canola oil in the feed as compared with the other sources (cotton oil, soybean oil, lard, sunflower oil).

**Table 4. Fatty acid composition of chicken grill oil and egg yolk as influenced by quail diets containing sunflower oil (SO) and chicken grill oil (CO).**

Item		Chicken grill oil	SO1	CO1	SO2	CO2
C14:0	Myristic	0.46	0.20	0.43	0.40	0.42
C16:0	Palmitic	20.29	27.09	28.28	26.10	27.66
C16:1	Palmitoleic	1.95	1.09	2.63	1.00	1.95
C18:0	Stearic	6.63	18.12	11.64	13.24	13.21
C18:1	Oleic (Omega 9)	30.63	32.38	43.24	39.10	37.61
C18:1(n:7)	Omega 7	1.68	1.10	2.01	1.10	1.40
C18:2	Linoleic Omega 6	35.11	16.42	9.58	17.00	15.24
C18:3	Linolenic	2.81	0.15	0.10	0.10	0.12
C20:4	Arachidonic (omega 6)	-	3.10	1.80	1.83	1.89

5% sunflower oil (SO1), 5% chicken grill oil (CO1), 7.5% sunflower oil (SO2), 7.5% chicken grill oil (CO2).

As a conclusion, this study showed that if chicken grill oil was collected and stored properly, dietary chicken grill oil had no adverse effect on egg weight, cholesterol and fatty acid composition of egg yolk and feed conversion ratio. Chicken grill oil can be use up to 7.5% of the laying quail diets.



## ACKNOWLEDGEMENTS

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## MYCOBIOTA OF SOILS FROM WHEAT GROWING AREAS OF THE CSONGRÁD-TIMIS REGION

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### ABSTRACT

Cereals can be attacked by several fungal pathogens, some of which may cause serious yield and quality losses. Besides, several fungal plant pathogens also contaminate cereals with various mycotoxins which are harmful to animals or humans. Several plant pathogenic fungi are soil-borne pathogens. We examined the mycobiota of soil collected in wheat growing areas in the Csongrád-Timis region to get insight into the role of soil in the occurrence of wheat diseases. Soil samples were collected from 10-10 fields in both countries at three different times. Samples were diluted and plated onto appropriate media to identify the fungi. Fungal identifications were carried out using morphological and molecular sequence-based methods. Based on the results, the soil samples contained large amounts of *Trichoderma* isolates. Besides, several potential mycotoxin producers have been identified, including black *Aspergillus* species which are potentially able to produce ochratoxins and fumonisins, *Aspergillus allicaues*, which is able to produce ochratoxins, several *Penicillium* species which can produce a range of mycotoxins, and several *Fusarium* species including the well-known cereal pathogens and mycotoxin producers *F. graminearum*, *F. oxysporum* and *F. sporotrichioides*. Among the cereal pathogens, *Pyrenophora teres* and *Cochliobolus lunatus* were also identified in the soil samples. We also identified *Clonostachys rosea* (teleomorph: *Bionectria ochroleuca*) in some of the soil samples. This species produces a range of enzymes which can be used for the biodegradation of zearalenone. This is the first report on its occurrence in Hungary.

**Keywords:** soil, wheat, sequence-based identification, mycobiota, *Aspergillus*, *Penicillium*, *Fusarium*

### INTRODUCTION

Agricultural soils are natural, living bodies, which change in space and time. The soils from the cross border region between Hungary and Romania play a major role in the long term sustainability of agriculture in the region. Soil is an important source of plant pathogens of cereals. Fungal pathogens of cereals can either be seedborne (e.g. *Ustilago* sp.), airborne or soilborne. However, most of the fungal pathogens of cereals are able to infect the plant from the soil (or plant debris present in the soil). The various fungal pathogens can cause serious yield and quality losses. Besides, several of these pathogens (e.g. *Fusarium*, *Penicillium* and *Aspergillus* species) also contaminate the cereals with various mycotoxins, secondary metabolites which cause various disease symptoms in animals and humans. The soil is also an important source of fungi which can be used in biocontrol strategies to combat plant diseases, or to lower the mycotoxin content of agricultural products. Prime examples of these useful fungi are *Trichoderma* species which can be used as biocontrol agents against various plant pathogens (KREDICS et al., 2003), or other fungal species which are important sources of mycotoxin degrading enzymes (VARGA and TÓTH, 2005).



In this study, we examined the mycobiota of soil in wheat growing fields in the Csongrád-Timis region to get insight into the role of soil in the occurrence of wheat diseases, and to identify potential candidates which could be used in biocontrol experiments to lower mycotoxin contamination of cereals.

## MATERIAL AND METHOD

### Collection of soil samples

Soil samples will be collected 3 times, in April, August and November, 2011 in 10 Hungarian and 10 Romanian sampling sites. Samples were collected in each site from two different depths. Seven test sites include wheat fields with intensive cultivation, while 1 site was selected from an organic wheat cultivation field in each country. Test sites including a pasture field and a forest were included as controls. Soils were sampled according to the soil sampling guidelines (90/2008. (VII. 18.) Regulation of Ministry of Rural Development).

### Sample analyses

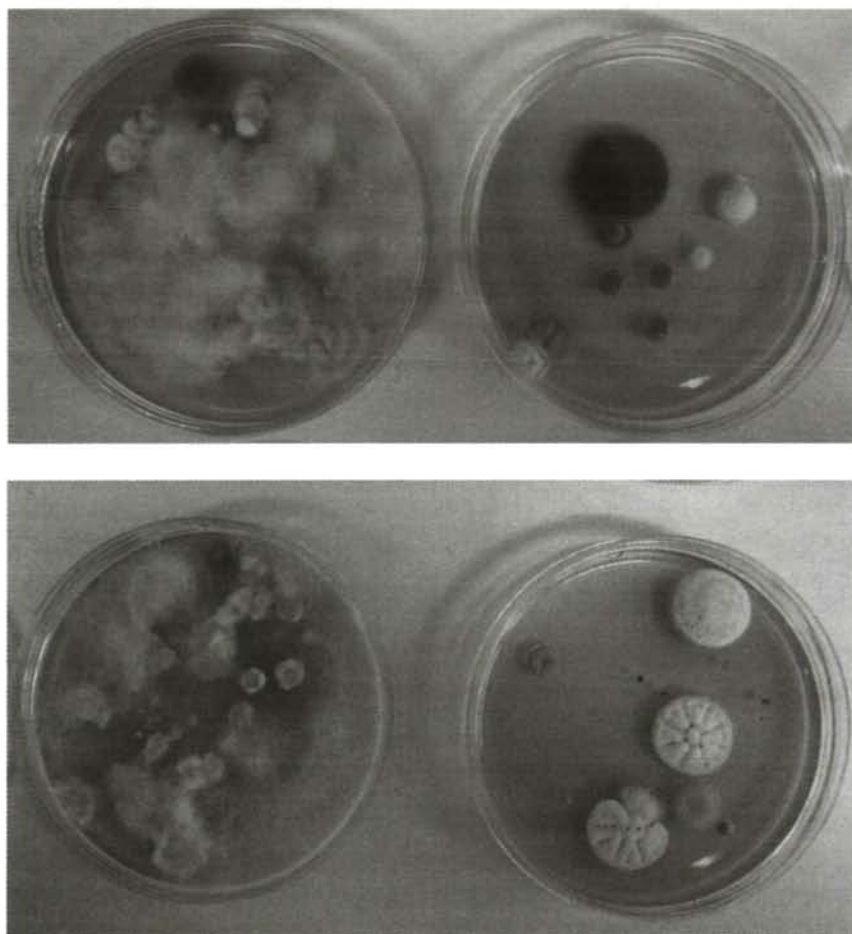
Appropriate dilutions of the samples were plated on selective media (Dichloran Rose Bengal Agar medium, DRBC, KING et al., 1979) and incubated at 25°C for 1-2 weeks. The colonies were transferred to malt extract agar (MEA) plates and purified. The growing colonies were examined using standard macro- and micromorphological methods (SAMSON et al., 2004). DNA extractions from selected isolates were carried out as described previously (SAMSON et al., 2007). Species assignment of the isolates was carried out using sequence analysis of appropriate gene fragments of the isolates. Usually, the ITS region was amplified and sequenced, while for the species assignment of *Aspergillus* and *Penicillium* species part of the calmodulin gene was used as target (SAMSON et al., 2007).

## RESULTS

The mycobiota of soil samples collected from wheat fields from 10-10 locations in the Csongrád-Timis region was examined using morphological and molecular methods. The number of fungal colonies isolated from the soil samples was in general much higher in August than in November or in April. Besides, the soil samples collected from the upper layers of soil were usually more contaminated by fungi than the lower layers (Figure 1). Several well-known soil-inhabiting fungi were identified. Among *Trichoderma* species, *T. hamatum*, *T. koningiopsis*, *T. virens*, *T. brevicompactum*, *T. gamsii* and *T. pleuroticola* were identified. The latter species has recently been described as an important pathogen of oyster mushrooms (KOMON-ZELAZOSWSKA et al., 2007). Our data indicate that a possible source of *T. pleuroticola* is the compost used for mushroom cultivation. Among *Fusaria*, the well-known cereal pathogens *F. graminearum* and *F. sporotrichioides* (Figure 2) were identified, which are producers of a variety of mycotoxins including trichothecenes (deoxynivalenol, nivalenol, T2-toxin) and zearalenone. Besides, *F. oxysporum* was also found in some of the samples.

*Aspergillus* species have also been identified. Among them, black *Aspergilli* including *A. niger* and *A. awamori* are potential producers of two carcinogenic mycotoxins, ochratoxins and fumonisins, *A. alliaceus* can produce ochratoxins, while *A. terreus* is able to produce a range of mycotoxins including citreoviridin, terrein and tremorgenic mycotoxins (SAMSON et al., 2011a). *Aspergillus calidoustus* (Figure 2) has

recently been described by our group (VARGA et al., 2008). This species is an important opportunistic human pathogen, and is a source of useful metabolites including ophiobolins (SAMSON et al., 2011b). To our knowledge, this is the first report on its occurrence in Hungary.

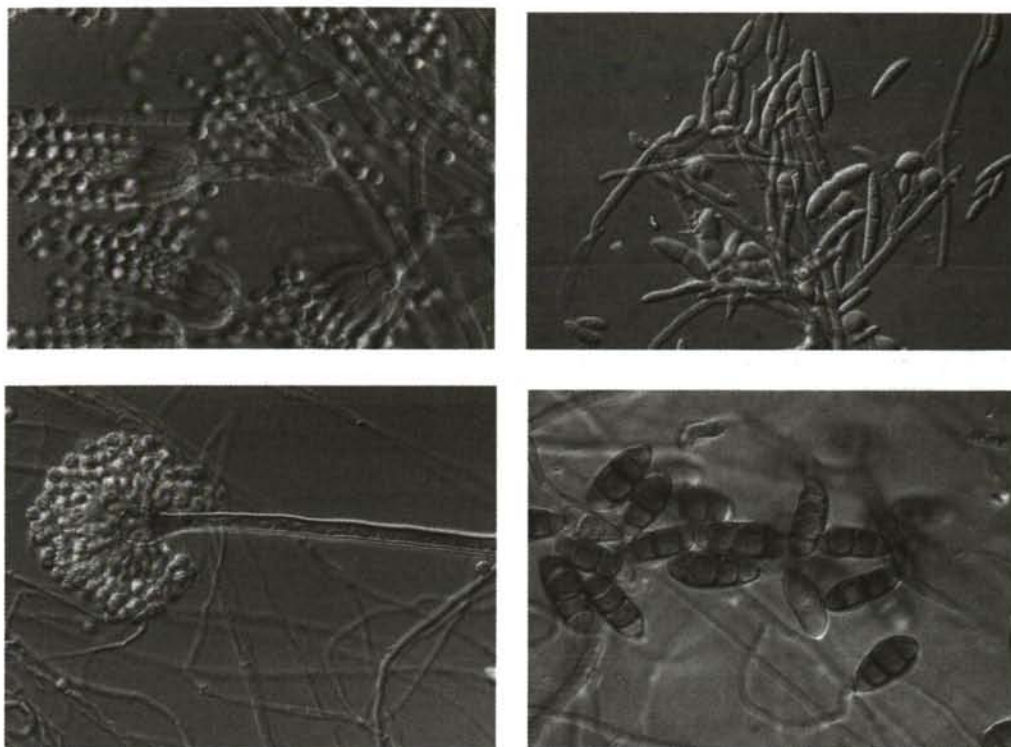


**Figure 1: Mycobiota of soil samples taken from the upper (left, 0-15 cm) and the lower (right, 15-30 cm) layers of soil in sampling sites located in Cenad (top) and Sándorfalva (bottom).**

In the *Penicillium* genus, most of the isolates were found to belong to the *P. verruculosum* species, which can produce some tremorgenic mycotoxins, and can live as an endophyte in the root system of some plants (BHAGOBATY et al., 2010). Other species identified include *P. glabrum* (Figure 2), *P. expansum* (a well-known producer of patulin, a toxigenic compound), *P. griseofulvum*, *P. janthinellum*, *P. angulare*, *P. manginii*, *P. anaticum* and *P. pinophilum* (recently transferred to *Talaromyces*; SAMSON et al., 2011c). Apart from the mycotoxin producing fungi, well-known plant pathogens including *Pyrenophora teres* and *Cochliobolus lunatus* (synonym: *Curvularia lunata*) have also been identified (Figure 2). *Pyrenophora teres* is an important pathogen of cereals especially barley, and it has recently been also been identified on wheat in Hungary (TÓTH et al., 2008).

Another interesting fungus, *Clonostachys rosea* (teleomorph: *Bionectria ochroleuca*) has also been identified for the first time in the soil samples. This species produces a range of enzymes which can be used for the biodegradation of zearalenone (KAKEYA et al., 2002; TAKAHASHI-ANDO et al., 2004).





**Figure 2: Microscopic pictures of some of the isolates. *Penicillium glabrum* (top left), *Fusarium sporotrichioides* (top right), *Aspergillus calidoustus* (bottom left), *Cochliobolus lunatus* (bottom right).**

## CONCLUSIONS

Examination of the mycobiota of wheat-growing areas in the Csongrád-Timis region revealed that soil serves as an important source of mycotoxigenic and plant pathogenic fungi in the region. At the same time, soil is also an important and invaluable source of potentially useful fungi which can be used in the biocontrol of plant pathogens, or as sources of enzymes and their genes to be used to detoxify important mycotoxins including zearalenone and others. Further studies are in progress to compare the mycobiota of the soil samples to those of the agricultural products cultivated on these fields.

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## **ANALYSIS OF ENVIRONMENTAL IMPACT ON SLOPE PROTECTION CONSTRUCTIONS AND ENVIRONMENTAL FRIENDLY SOLUTIONS OF SLOPE PROTECTION REHABILITATION**

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### **ABSTRACT**

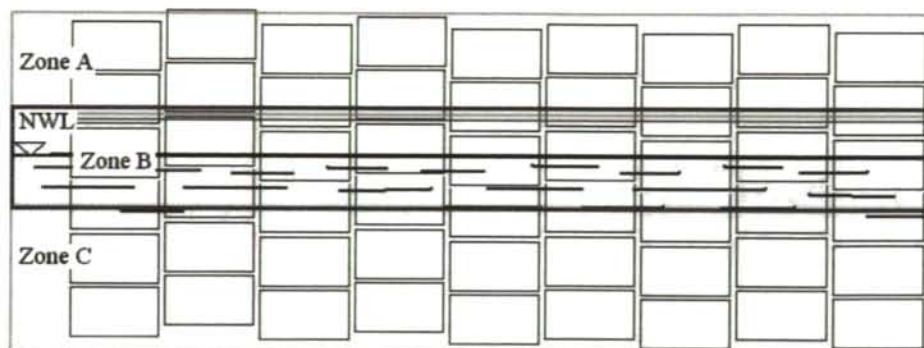
There were investigated 32 Lithuanian earth dam slopes protected with concrete in the years 1998–2011. The purpose of these investigations, based on research in the field, is to evaluate the impacts of environment on reinforced concrete slabs for earth dam slope protection by establishing character and causes of the main deteriorations and ruptures in the slabs. Further goal is to evaluate frost impact on the change of concrete properties and durability of covering layer of the slabs and to suggest environmental friendly solutions of slope protection rehabilitation. During expeditions the structures were visually examined on location and their most deteriorated places were detected, typical defects and deteriorations were measured. Furthermore, regularities of concrete compression strength and water absorbability of reinforced concrete slabs for earth dam slope protection under the influence of freezing–thawing cycles and other environmental factors were determined. Using the durability evaluation method of reinforced concrete slabs for earth dam slope protection created by us, the period left to use the construction till the probable deterioration start ( $T_{5\%}$ ) or end ( $T_{50\%}$ ) may be calculated. Environmental friendly solution for the rehabilitation of slope protection structures is proposed.

**Keywords:** reinforced concrete slabs, frost cycles, concrete compression strength, durability

### **INTRODUCTION**

There are over 1100 dams in Lithuania presently, that have created ponds bigger than 0.5ha with. Water accumulated in ponds presents a danger for the community and the environment both by the water head and by the accumulated water volume. In Lithuania there are 617 ponds evaluated as potentially dangerous hydraulic structures. The reinforced concrete strengthening slabs (hereinafter - RCSS) are the most popular main coverage of slopes. The monolithic, precast or combine (cast in place - precast) reinforced concrete slabs were used. The reinforced concrete coverage of earth dams were divided in separate section by contraction joints. The covering of earth dams slopes was constructed and arranged in accordance with building standards and regulations. Reinforced concrete strengthening slab constructions are exploited in rather hard conditions. They are under the impacts of various loads and aggressive environment: atmosphere, water medium (surroundings), ice, etc. The main slope coverage is in the most intensive ice and water waves impact zone B (*Figure 1*), the reinforced concrete slabs for earth dam slope protection deteriorate and lose their durability.





**Figure 1. The zones of reinforced concrete coverage of earth dams**

Durability depends on the maintenance circumstances, surveillance, timely repair of structures or reconstructions. The reinforced concrete is a durable material, but like any other one it is deteriorated in time. Many hydraulic structures on hydroschemes in Lithuania are older than 30 years, therefore the ageing of building materials causes greater probability of deterioration. Deteriorations of constructions create not only favorable conditions for rapid destruction of the structure, but can cause crash of the whole construction as well. Evaluation of environmental factors [freezing–thawing (hereinafter – frost) cycles, ice loads] and the deterioration process (erosion, construction defects and deterioration development) speed of slabs caused by them, and slabs durability prognosis is the main problem. Level of its research is inadequate.

**Frost cycles.** Analysis of various researchers' works (RAMONAS, 1995; GURSKIS, 1996) about the influence of frost cycles on the concrete of hydraulic structures showed, that so far, in the scientific literature the method for the determination of slab durability based on the evaluation of change of main physical–mechanical properties of the concrete under the influence of frost cycles is not sufficiently discussed.

**Defects and deteriorations.** For the classification of defects and deterioration of civil, industrial and hydraulic structures, for their state evaluation there is paid much attention in Lithuanian and foreign scientists' works (КАВЕШНИКОВ, 1989; KAMAITIS, 2000; JOKŪBAITIS AND KAMAITIS, 2000; VAIŠVILA ET AL., 2001). The state of structures and constructions is evaluated according to the main indices of defects and deterioration and is expressed by the method of grades (points, sorts, categories). Analysis of the literature and the currently used building standards and regulations shows that archetypical defects and technical state of RCSS is not described very strictly. There is a lack of information about the impact of defects on the durability of these structures.

The purpose of these investigations, based on research in the field, is to evaluate the impacts of environment on reinforced concrete slabs for earth dam slope protection by establishing character and causes of the main deteriorations and ruptures in the slabs. Further goal is to evaluate frost impact on the change of concrete properties and durability of the covering layer of the slabs and to suggest environmental friendly solutions of slope protection rehabilitation.

## **MATERIAL AND METHOD**

There were investigated 32 earth dam slopes protected with reinforced concrete slabs for the years 1998–2011. The structures were visually examined on location and their most deteriorated places were established, typical defects and deteriorations were measured. By



the field investigations and laboratory tests (by standard methods) the main physical-mechanical properties of RCSS – their concrete compression strength and water absorbability were determined and statistically evaluated. The compression strength of concrete structures was estimated by nondestructive and destructive methods in accordance with the standard and instructional manual of the instrument devices.

By the statistically evaluated research results of RCSS, concrete compression strength and water absorbability were calculated rates of concrete resistance to frost. We used a new, nonstandard concrete frost resistance mark determination method worked out by the employees of the Department of Building Constructions at the Lithuanian University of Agriculture (hereinafter – Dept. of Building Constructions), where this property is approximately evaluated by concrete compression strength and water absorbability (VAIŠVILA ET AL., 2002; VAIŠVILA ET AL., 2003; VAIŠVILA ET AL., 2004).

Knowing compression strength  $f_c$  of the concrete (LST EN 12504-2:2003, LST EN 12390-3:2003-12), water absorbability by mass  $W_m$  (LST 1428.18:1997) and allowed or forecasted loss of the concrete strength  $\Delta f_c$ , it is possible to estimate laboratory frost cycles number  $n_{50}$  (ГОСТ 10060–87):

$$n_{50} = c \cdot \Delta f_c^{-d}, \quad (1)$$

where

$n_{50}$  – numbers of laboratory frost cycles freezing samples until  $-55 \pm 2^\circ \text{C}$  by ГОСТ 10060–87,

$\Delta f_c$  – concrete strength loss in % due the influence of frost cycles, calculated by LST 1428.17:2005,

$c, d$  – coefficients found in the tables made by employees of Dept. of Building Constructions (VAIŠVILA et al, 2002).

As far as concrete frost resistance mark  $F$  shows the number of frost cycles  $n_f$  when samples are freezed in  $-18 \pm 2^\circ \text{C}$ , the number of standard cycles is calculated by Dept. of Building Constructions employees' formula (VAIŠVILA ET AL., 2004):

$$n_F = 34.848 \cdot n_{50}^{0.6157}. \quad (2)$$

Evaluating the impact of frost cycles on the change of investigated RCSS concrete physical-mechanical properties the frost resistant indices  $F_{50\%}$  were used.

According to the construction regulations (Рекомендации ..., 1990), deterioration end was fixed by the number of cycles  $F_{50\%}$ , where the concrete compression strength of the 25–30 mm thick outer concrete layer (hereinafter – covering) shrinks twice, i.e. to 50% of the estimated strength during the research.

Natural frost cycles, by means of corrective coefficients (chosen by Dept. of Building Constructions employees made graph), were recounted into laboratory freezing–thawing cycles (from  $-18^\circ \text{C}$  to  $+18^\circ \text{C}$ ) and further recalculated to the indices of structures durability – probable deterioration end time  $T_{50\%}$ .

## RESULTS

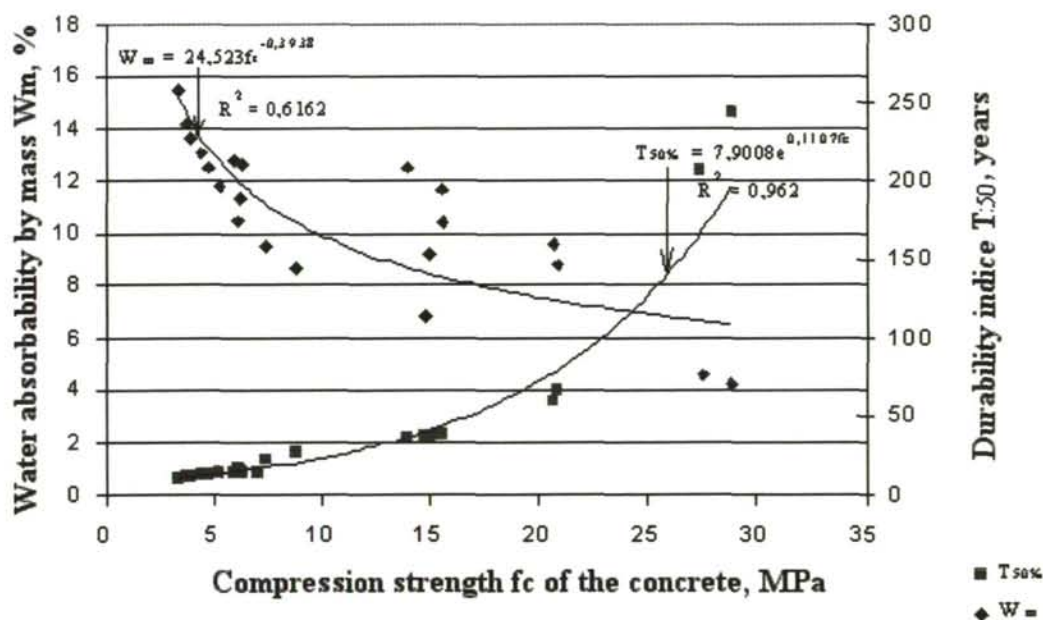
At present time the technical state of reinforced concrete structures of functioning hydraulic structures in Lithuania is not the same. There is a number of earth dam slope covering structures being in almost good condition, others are less or more deteriorated. The results of field investigations of 32 earth dam slope protection slabs show that the mostly occurred defects and deteriorations of slab were: deterioration of cover layer (11 from 32 objects) and collapsing of junctures (16 from 32 objects) at 34% and 50% of the researched objects, respectively. It was established, that cover layer and juncture defects are caused by the environmental (frost cycles; ice, wave blows; moss, grass, bushes roots,



collapsing impacts; periodical wetting, etc.) impacts, appearing in degradation processes (concrete and reinforcement corrosion, erosion, biological actions). It was noticed during the expedition, that RCSS are mostly eroded by the ice thermal expansion load.

Deterioration processes mostly break the badly made covering layer (small concrete strength and frost resistance) which, being under the influence of frost cycles, crumbles. Its physical-mechanical properties change, form deteriorations, pitting. Most intensively, the concrete is destroyed due to the impact of ice and waves (changing water level) on zone B. Also main attention must be paid to the zones where deterioration and defects are often formed – pitting is formed in the changing water level in flow compression zone: the inflow part of shaft spillways or in the flow parts of overflow spillways. The rate of erosion is dependent on a number of factors including the size, shape, quantity, and hardness of particles being transported, the velocity of the water, and the quality of the concrete. While high-quality concrete is capable of resisting high water velocities for many years with little or no damage, the concrete cannot withstand the abrasive action of debris grinding or repeatedly impacting on its surface. In such cases, abrasive erosion, ranging in depth from a few millimeters to a meter or more, can happen depending on the flow conditions. There are several reasons (mentioned above) forming pitting, but we focused the main attention on the erosion of concrete by the influence of frost cycles (ŠADZEVIČIUS, 2007).

By the statistically evaluated research results of RCSS, concrete compression strength and water absorbability were calculated rates of concrete resistance to frost and further recalculated to the indices of structures durability – probable starting ( $T_5$  %) and ending time ( $T_{50}$  %) of deterioration. Relationship between durability indices  $T_{50\%}$  and average compression strength of concrete  $f_c$  and water absorbability  $W_m$  is shown in Figure 2.



**Figure 2. Relationship between durability indices  $T_{50\%}$  and average compression strength of concrete  $f_c$  and water absorbability  $W_m$**

The probable deterioration end time  $T_{50\%}$  (covering layer strength  $f_c$  loss under the frost influence by 50%) may be expressed by

$$T_{50\%} = 7,9008 e^{0,1107f_c}, \quad (3)$$

To illustrate how fast concrete covering layer losing the strength under the frost influence



were calculated indices of structures durability – probable deterioration end time  $T_{50}$  in Grauzes hydroscheme (Kaunas distr.). The calculation results are shown in *Table 1*.

**Table 1. The calculation results of durability indices based on determined and statistically evaluated main physical–mechanical properties of RCSS**

Date	2011.04				
Indices	$f_c$ , MPa $W_m$ , %	$F_{5\%}$ , cycles	$F_{50\%}$ , cycles	$T_{5\%}$ , years	$T_{50\%}$ , years
Zone B	18.0 7.7	66.2	249.7	13.2	50.0
Zone A	30.4 5.6	113.8	558.3	22.8	111.7

$f_c$ : compression strength of the concrete;

$W_m$ : water absorbability by mass;

$F_{5\%}$  and  $F_{50\%}$ : number of cycles, where the concrete compression strength of the 25–30 mm thick concrete covering layer shrinks 5% and 50%;

$T_{5\%}$  and  $T_{50\%}$ : the period left to use the construction till the probable deterioration start or end, respectively.

According to the data presented, a 30 mm covering layer made of weaken concrete ( $f_c = 18.0$  MPa) will be destroyed in 50.0 years of functioning, while the one made of stronger concrete ( $f_c = 30.4$  MPa) will be destroyed in 111.7 years of functioning. As it can be seen in *Table 1*, concrete is weakening under ice and waves impact (changing water level) in zone B, so this zone should be protected with structure of higher strength, or should be protected with environmental friendly solutions of slope protection.

Usually, in Lithuania streambank protection methods involve such rigid structural approaches as rip-rap, concrete, dikes, fences, asphalt, gabions, matting, and bulkheads which, directly and indirectly, have influenced the characteristics of riparian areas.

In the last years, the application of bioengineering techniques has favoured the planning of protective structures with a lowered environmental impact: sunken fascine rolls, anterior stakes, wattle work, live brush mattresses, brushlayers, crib groynes with cuttings (living groynes), etc. Today in Lithuania has just been started the application of one of the environmental friendly methods, the earth dam slope protection using flexible solutions – geocells (geogrids).

## CONCLUSIONS

The results of field investigations of 32 earth dam slope protection slabs show that the mostly occurring defects and deteriorations of slabs are: deterioration of cover layer and collapsing of junctures at 30% and 50% of the researched objects, respectively.

The influence of frost on the change of physical–mechanical properties and on the durability of covering layer of reinforced concrete slabs was evaluated. By means of the evaluation, knowing values of structures concrete strength  $f_c$ , water absorbability  $W_m$  and allowed or forecasted loss of the concrete strength  $\Delta f_c$ , using function (3) the main indices of reinforced concrete slab durability can be calculated – the probable deterioration end time  $T_{50\%}$  (covering layer strength  $f_c$  loss under the frost influence by 50%).

Using function (3) for designed reinforced concrete strengthening slabs which are on the changing water level, concrete of such strength can be chosen, that reinforcement of these structures will not uncover during the foreseen time.

For the rehabilitation of slope protection structures in Lithuania, it is suggested to use environmental friendly solutions such as geocells (geogrids).

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